04/07/00
jc780 U.S. PTO

UTILITY PATENT APPLICATION TRANSMITTAL **(Large Entity)** *(Only for new nonprovisional applications under 37 CFR 1.53(b))*	Docket No. 12582
	Total Pages in this Submission **3**

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

ANTICANCER CALCIUM CHANNEL BLOCKERS

U.S. PTO
09/544963
jc520   04/07/00

and invented by:

Lloyd S. Gray
Timothy L. Macdonald
Doris M. Haverstick
Tiffany N. Heady

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☐ **Continuation**   ☐ **Divisional**   ☐ **Continuation-in-part (CIP)**   of prior application No.: _____

Which is a:

☐ **Continuation**   ☐ **Divisional**   ☐ **Continuation-in-part (CIP)**   of prior application No.: _____

Which is a:

☐ **Continuation**   ☐ **Divisional**   ☐ **Continuation-in-part (CIP)**   of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below

2. ☒ Specification having _____ 78 _____ pages and including the following:

   a. ☒ Descriptive Title of the Invention

   b. ☒ Cross References to Related Applications *(if applicable)*

   c. ☐ Statement Regarding Federally-sponsored Research/Development *(if applicable)*

   d. ☐ Reference to Microfiche Appendix *(if applicable)*

   e. ☒ Background of the Invention

   f. ☒ Brief Summary of the Invention

   g. ☒ Brief Description of the Drawings *(if drawings filed)*

   h. ☒ Detailed Description

   i. ☒ Claim(s) as Classified Below

   j. ☒ Abstract of the Disclosure

P01ULRG/REV04

<table>
<tr><td colspan="2"><h1>UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)</h1>(Only for new nonprovisional applications under 37 CFR 1.53(b))</td><td>Docket No.
12582</td></tr>
<tr><td></td><td></td><td>Total Pages in this Submission
3</td></tr>
</table>

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*

    a. ☐ Formal   Number of Sheets _____

    b. ☒ Informal   Number of Sheets _____42_____

4. ☐ Oath or Declaration

    a. ☐ Newly executed *(original or copy)*   ☐ Unexecuted

    b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*

    c. ☐ With Power of Attorney   ☐ Without Power of Attorney

    d. ☐ *DELETION OF INVENTOR(S)*
       Signed statement attached deleting inventor(s) named in the prior application,
       see 37 C.F.R. 1.63(d)(2) and 1.33(b).

5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
    The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Computer Program in Microfiche *(Appendix)*

7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*

    a. ☐ Paper Copy

    b. ☐ Computer Readable Copy *(identical to computer copy)*

    c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*

9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*

10. ☐ English Translation Document *(if applicable)*

11. ☐ Information Disclosure Statement/PTO-1449   ☐ Copies of IDS Citations

12. ☐ Preliminary Amendment

13. ☒ Acknowledgment postcard

14. ☒ Certificate of Mailing

    ☐ First Class   ☒ Express Mail *(Specify Label No.):* EL357933246US

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UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)
(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
12582

Total Pages in this Submission
3

Accompanying Application Parts (Continued)

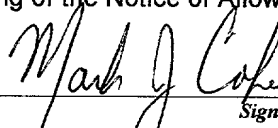15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Additional Enclosures *(please identify below)*:

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	115	- 20 =	95	x $18.00	$1,710.00
Indep. Claims	2	- 3 =	0	x $78.00	$0.00
Multiple Dependent Claims (check if applicable) ☒					$260.00
				BASIC FEE	$690.00
OTHER FEE *(specify purpose)*					$0.00
				TOTAL FILING FEE	$2,660.00

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  - ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance,
    pursuant to 37 C.F.R. 1.311(b).

_____
Signature

Mark J. Cohen
Registration No.: 32,211

Dated: **April 7, 2000**

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CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)	Docket No.
Applicant(s): Llyod S. Gray, et al.	12582

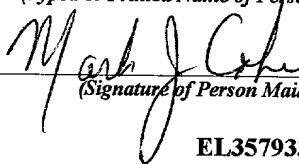| Serial No. Unassigned	Filing Date Herewith	Examiner Unassigned	Group Art Unit Unassigned

Invention: **ANTICANCER CALCIUM CHANNEL BLOCKERS**

I hereby certify that this <u>**New Patent Application**</u>

(Identify type of correspondence)

is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under

37 CFR 1.10 in an envelope addressed to: The Assistant Commissioner for Patents, Washington, D.C. 20231

on _____**April 7, 2000**_____

(Date)

Mark J. Cohen

(Typed or Printed Name of Person Mailing Correspondence)

(Signature of Person Mailing Correspondence)

EL357933246US

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ANTICANCER CALCIUM CHANNEL BLOCKERS

RELATED APPLICATION

This application claims benefit from U.S. Provisional Application No. 60/128,143, filed April 7, 1999.

FIELD OF THE INVENTION

The present invention relates to novel compounds useful as cancer cell inhibitors, compositions containing such compounds, methods for inhibiting calcium entry into electrically non-excitable cells as well as methods for preventing proliferation of electrically non-excitable cells.

BACKGROUND OF THE INVENTION

Anti-metabolic, cytotoxic therapies for cancer have achieved success in extending the lives of people afflicted with this disease. The goal of this approach to cancer treatment, at its limit, is the complete eradication of cancer cells. Elimination of all residual cancer cells results in cure, although emergence of drug resistance or of more aggressive disease often hampers this outcome. The goal of cytostatic cancer therapies is to retard cellular proliferation rather than eliminate all cancer cells. Controlling the growth of cancer would, at one extreme, effectively render the disease impotent. Failure to completely control the growth of cancer would nonetheless be clinically valuable if, for example, cytostatic therapy significantly extended the duration of remissions induced by cytotoxic agents.

Malignant transformation is often associated with the acquisition of a phenotype that is consistent with an abnormally high sensitivity to ambient

concentrations of growth factors. In prostate cancer, for example, the source of such growth factors can be autocrine, from the cancer cells themselves, or from the surrounding stroma in a paracrine fashion (Russel et al. (1998) Clin. Chem. 44(4):705-723, Steiner, M.S. (1993) Urology 42:99-110). The molecular role of growth factors and their corresponding receptors in malignant transformation and cancer progression is complex and not yet well understood.

Growth factor receptors are often linked to the pathway that regulates calcium homeostasis. The mitogenic interaction of a growth factor with its receptor can activate a pathway that includes enhancement of the entry of extracellular Ca^{2+}. Engagement of a growth factor receptor by an appropriate ligand results in the activation of phospholipase C by tyrosine phosphorylation (Exton, J.H. Ann. Rev. Pharmacol. Toxicol. 36:481-509). Activated phospholipase C metabolizes phosphatidyl inositol bisphosphate to produce diacylglycerol and inositol 1,4,5-triphosphate (Berridge et al.(1984) Nature 312:315-321). Inositol triphosphate releases Ca^{2+} from an internal storage depot, and this release of intracellular Ca^{2+} triggers the influx of extracellular Ca^{2+} (Berridge, supra).

The role of enhanced Ca^{2+} entry in the proliferation of cancer cells is not well understood. It has been shown, however, that proliferation of at least some cancer cell lines can be slowed or stopped at specific points in the cell cycle by removal of extracellular Ca^{2+}. (Meldolesi, J. (1995) Nat. Med. 1:512-513; Alessandro et al. (1996) In Vivo 10:153-160). Consistent with this observation is that a drug that

-2-

blocks Ca^{2+} entry can retard the metastasis of human melanoma cells in immune deficient mice (Benzaquen et al. (1995) <u>Nat</u>. <u>Med</u>. 1:534-540).

While the role of Ca^{2+} entry in cancer cell proliferation has been known for some time, the use of directed Ca^{2+} entry antagonists for the suppression of Ca^{2+} influx and treatment of cancer had not been developed until now. For the first time and in accordance with the present invention, compounds have been developed that block growth factor receptor-linked Ca^{2+} entry and growth factor-driven cellular proliferation both <u>in vitro</u> and <u>in vivo</u>. The compounds of the present invention are useful for inhibiting Ca^{2+} entry into and proliferation of cancer cells, such as breast and prostate cancer cells, without apparent toxicity.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to novel compounds useful for retarding the proliferation of cancerous cells and having the formula:

I
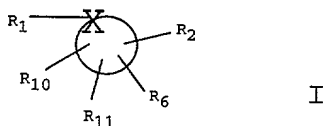
and pharmaceutically acceptable salts thereof wherein
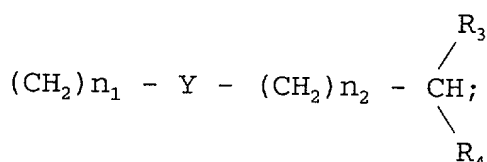
X is N or CH;

 is a 5-10 membered cyclic ring which is

saturated and which may contain 1 or 2 additional ring heteroatoms selected from the group consisting of O, S and N, with the remaining ring atoms being carbon atoms;

-3-

R_1 is $(CH_2)n - Z - (R_5)$, Q, hydrogen or lower alkyl;

R_2 is hydrogen or Q', provided that R_1 is Q or R_2 is Q';

Q and Q' may be the same or different and are independently

$$(CH_2)n_1 - Y - (CH_2)n_2 - \overset{\displaystyle R_3}{\underset{\displaystyle R_4}{CH}};$$

Z is a chemical bond, CH_2, O, S or NH;

Y is CH_2, O, S or NH;

R_3, R_4 and R_5 are independently cyclic rings containing 6-14 ring carbon atoms, and containing no hetero ring atoms, which cyclic rings may be completely saturated, partially unsaturated or aromatic, and which are unsubstituted or substituted with an electron donating group or electron withdrawing group; or

R_3 and R_4 may be fused to form a cyclic ring structure containing 12-28 carbon atoms;

R_{10}, R_6 and R_{11} are independently hydrogen or lower alkyl, which is unsubstituted or substituted with an electron withdrawing group or electron donating group;

n_2 is 0 to 8; and

n_2 and n_1 are independently 1-8.

These compounds are calcium antagonists and are effective calcium channel blockers.

The present invention is also directed to pharmaceutical compositions containing a pharmaceutically effective amount of these compounds and a pharmaceutical carrier therefor. The present invention is also directed to treating cancer in a mammal afflicted therewith

-4-

comprising administering to said mammal a cytostatic effective amount of said compound. The present invention is also directed to a method for inhibiting cancer cell proliferation in a mammal in need of such treatment comprising administering to said mammal a cytostatic effective amount of said compound. The present invention is also directed to a method for retarding the entry of calcium into electrically non-excitable cells of a mammal comprising administering to said mammal an amount of said compound effective to retard calcium absorption into electrically non-excitable cells of said mammal.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the chemical synthesis of TH-1177.

Figure 2 graphically illustrates the effect of TH-1177 on Ca^{2+} entry stimulated by thapsigargin in LNCaP cells. LNCaP cells were stimulated with 300 nM thapsigargin to initiate Ca^{2+} entry in a receptor independent manner. The indicated concentrations of TH-1177 were added prior to stimulation with thapsigargin (panel A) or after the influx pathway had been opened (panel B).

Figure 3 graphically illustrates the effect of TH-1177 on Ca^{2+} entry stimulated by ATP in LNCaP cells. The effect of 10µM TH-1177 on Ca^{2+} entry stimulated in a receptor dependent manner by 1 mM ATP was examined with TH-1177 added prior to stimulation with ATP (panel A) or after the influx pathway had been opened (panel B).

Figure 4 graphically illustrates the effect of TH-1177 on Ca^{2+} entry stimulated by ATP in PC3 cells. The effect of the indicated concentrations of TH-1177 on Ca^{2+} entry stimulated in a receptor dependent manner by 1 mM

ATP was examined with TH-1177 added prior to stimulation with ATP (panel A) or after the influx pathway had been opened (panel B).

Figure 5 graphically illustrates the effect of TH-1177 on release of Ca^{2+} from the internal storage depot. The effect of TH-1177 on IP3 mediated release of Ca^{2+} from the internal storage pool was monitored by chelating extracellular Ca^{2+} with the addition of EGTA to uncover the release component. The indicated concentrations of TH-1177 were added at 30s, followed by 2.5 mM EGTA at 60s and receptor stimulation with 1 mM ATP at 90s. Panel A: PC3 cells. Panel B: LNCaP cells.

Figure 6 graphically illustrates the effect of TH-1177 on cellular proliferation. LNCaP cells (panel A) at $2.5x10^4$ cells in a dilution of 100:1 or PC3 cells (panel B) at $5x10^4$ cells in a dilution of 100:1 were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1177. Results are the mean of 4 determinations.

In **Figure 7,** LNCaP cells were plated at $2.5x10^5$ cells per ml in the absence of drug or with 1μM (panel A) or 3μM (panel B) TH-1177 on day 1 in triplicate flasks and the number of viable cells determined in each flask on days 2 through 5. On days, 2, 3, and 4, all flasks were centrifuged and fresh media added with or without TH-1177 as indicated. The effect on cell growth in each circumstance is graphically depicted.

In **Figure 8,** LNCaP cells were grown in the absence (naive cells) or presence (prior treatment) of the indicated concentrations of TH-1177 for 48 hours. The media was removed and drug-free media was added for 48 hours. The drug-free media was then removed and media

-6-

containing drug was added to all cells for 72 hours. The
data, shown graphically, indicate the relative number of
cells at the end of this process.

Figure 9 tabulates and graphically illustrates
results of animal trials with TH-1177. SCID mice were
inoculated with $1x10^6$ PC3 cells on day 1. Mice received
daily injections of vehicle (no drug, open circles) or
TH-1177 at 3 mg/kg (closed circles) or 10 mg/kg (open
squares). The survival curve for this group of animals
is shown.

Figure 10 tabulates and graphically illustrates
the effect of TH-1087 on cellular proliferation of Jurkat
cells. Jurkat cells at 5×10^4/ml in 100μl were grown
for 48 hours in the absence (100% cell growth) or
presence of the indicated concentrations of TH-1087.
Results are the mean of 6 determinations.

Figure 11 tabulates and graphically illustrates
the effect of TH-1087 on cellular proliferation of PC3
cells. PC3 cells at 5×10^4/ml in 100μl were grown for
48 hours in the absence (100% cell growth) or presence of
the indicated concentrations of TH-1087. Results are the
mean of 6 determinations.

Figure 12 tabulates and graphically illustrates
the effect of TH-1087 on cellular proliferation of LNCaP
cells. LNCaP cells at 2.5×10^4/ml in 100μl were grown
for 48 hours in the absence (100% cell growth) or
presence of the indicated concentrations of TH-1087.
Results are the mean of 6 determinations.

Figure 13 tabulates and graphically illustrates
the effect of TH-1087 on cellular proliferation of MDA-
468 cells. MDA-468 cells at 5×10^4/ml in 100μl were
grown for 48 hours in the absence (100% cell growth) or

presence of the indicated concentrations of TH-1087. Results are the mean of 6 determinations.

Figure 14 tabulates and graphically illustrates the effect of TH-1087 on cellular proliferation of MDA-361 cells. MDA-361 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1087. Results are the mean of 6 determinations.

Figure 15 tabulates and graphically illustrates the effect of TH-1113 on cellular proliferation of Jurkat cells. Jurkat cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1113. Results are the mean of 6 determinations.

Figure 16 tabulates and graphically illustrates the effect of TH-1113 on cellular proliferation of PC3 cells. PC3 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1113. Results are the mean of 6 determinations.

Figure 17 tabulates and graphically illustrates the effect of TH-1113 on cellular proliferation of LNCaP cells. LNCaP cells at 2.5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1113. Results are the mean of 6 determinations.

Figure 18 tabulates and graphically illustrates the effect of TH-1113 on cellular proliferation of MDA-468 cells. MDA-468 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1113. Results are the mean of 6 determinations.

Figure 19 tabulates and graphically illustrates the effect of TH-1113 on cellular proliferation of MDA-361 cells. MDA-361 cells at 5 x 10^4/ml cells in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1113. Results are the mean of 6 determinations.

Figure 20 tabulates and graphically illustrates the effect of TH-1211 on cellular proliferation of Jurkat cells. Jurkat cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1211. Results are the mean of 6 determinations.

Figure 21 tabulates and graphically illustrates the effect of TH-1211 on cellular proliferation of PC3 cells. PC3 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1211. Results are the mean of 6 determinations.

Figure 22 tabulates and graphically illustrates the effect of TH-1211 on cellular proliferation of LNCaP cells. LNCaP cells at 2.5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1211. Results are the mean of 6 determinations.

Figure 23 tabulates and graphically illustrates the effect of TH-1211 on cellular proliferation of MDA-468 cells. MDA-468 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1211. Results are the mean of 6 determinations.

Figure 24 tabulates and graphically illustrates the effect of TH-1211 on cellular proliferation of MDA-

361 cells. MDA-361 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1211. Results are the mean of 6 determinations.

Figure 25 tabulates and graphically illustrates the effect of TH-1205 on cellular proliferation of Jurkat cells. Jurkat cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1205. Results are the mean of 6 determinations.

Figure 26 tabulates and graphically illustrates the effect of TH-1205 on cellular proliferation of PC3 cells. PC3 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1205. Results are the mean of 6 determinations.

Figure 27 tabulates and graphically illustrates the effect of TH-1205 on cellular proliferation of LNCaP cells. LNCaP cells at 2.5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1205. Results are the mean of 6 determinations.

Figure 28 tabulates and graphically illustrates the effect of TH-1205 on cellular proliferation of MDA-468 cells. MDA-468 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of TH-1205. Results are the mean of 6 determinations.

Figure 29 tabulates and graphically illustrates the effect of TH-1205 on cellular proliferation of MDA-361 cells. MDA-361 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or

presence of the indicated concentrations of TH-1205. Results are the mean of 6 determinations.

Figure 30 tabulates and graphically illustrates the effect of MMR-64 on cellular proliferation of Jurkat cells. Jurkat cells at 5×10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-64. Results are the mean of 6 determinations.

Figure 31 tabulates and graphically illustrates the effect of MMR-64 on cellular proliferation of PC3 cells. PC3 cells at 5×10^4/ml in 100µl were grown for 48 hours in the absence (100% growth) or presence of the indicated concentrations of MMR-64. Results are the mean of 6 determinations.

Figure 32 tabulates and graphically illustrates the effect of MMR-64 on cellular proliferation of LNCaP cells. LNCaP cells at 2.5×10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-64. Results are the mean of 6 determinations.
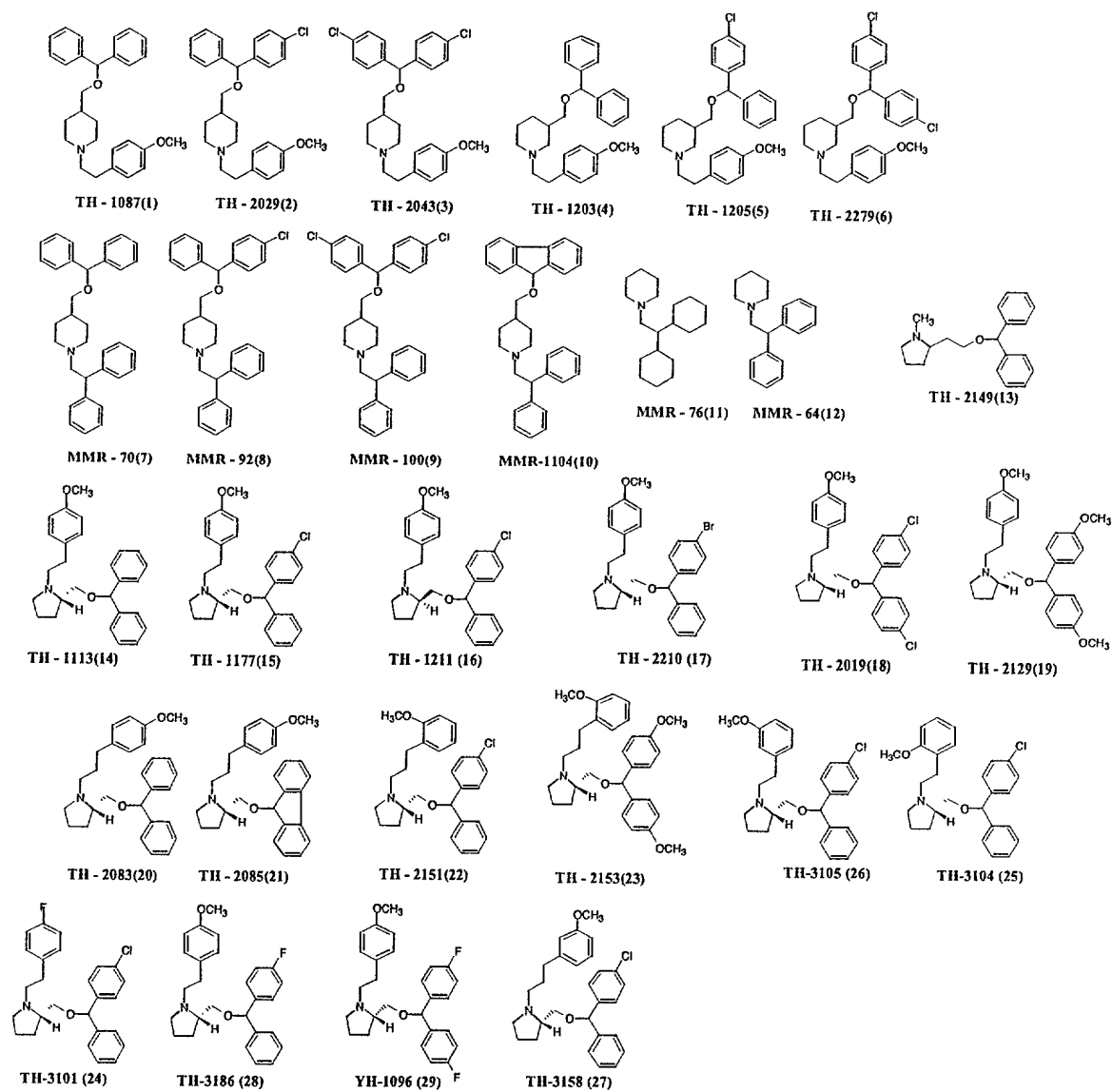
Figure 33 tabulates and graphically illustrates the effect of MMR-64 on cellular proliferation of MDR-468 cells. MDR-468 cells at 5×10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-64. Results are the mean of 6 determinations.

Figure 34 tabulates and graphically illustrates the effect of MMR-64 on cellular proliferation of MDA-361 cells. MDA-361 cells at 5×10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-64. Results are the mean of 6 determinations.

Figure 35 tabulates and graphically illustrates the effect of MMR-70 on cellular proliferation of Jurkat cells. Jurkat cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-70. Results are the mean of 6 determinations.

Figure 36 tabulates and graphically illustrates the effect of MMR-70 on cellular proliferation of PC3 cells. PC3 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-70. Results are the mean of 6 determinations.

Figure 37 tabulates and graphically illustrates the effect of MMR-70 on cellular proliferation of LNCaP cells. LNCaP cells at 2.5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-70. Results are the mean of 6 determinations.

Figure 38 tabulates and graphically illustrates the effect of MMR-70 on cellular proliferation of MDA-468 cells. MDA-468 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-70. Results are the mean of 6 determinations.

Figure 39 tabulates and graphically illustrates the effect of MMR-70 on cellular proliferation of MDA-361 cells. MDA-361 cells at 5 x 10^4/ml in 100µl were grown for 48 hours in the absence (100% cell growth) or presence of the indicated concentrations of MMR-70. Results are the mean of 6 determinations.

Figure 40 graphically illustrates the correlation of inhibition of Calcium Influx and Proliferation in LNCaP cells by exemplary compounds of the present invention. The legend respecting the compounds are as indicated hereinbelow:

TH - 1087(1) TH - 2029(2) TH - 2043(3) TH - 1203(4) TH - 1205(5) TH - 2279(6)

MMR - 70(7) MMR - 92(8) MMR - 100(9) MMR-1104(10) MMR - 76(11) MMR - 64(12) TH - 2149(13)

TH - 1113(14) TH - 1177(15) TH - 1211 (16) TH - 2210 (17) TH - 2019(18) TH - 2129(19)

TH - 2083(20) TH - 2085(21) TH - 2151(22) TH - 2153(23) TH-3105 (26) TH-3104 (25)

TH-3101 (24) TH-3186 (28) YH-1096 (29) TH-3158 (27)

-14-

Figure 41 graphically shows the ability of TH-1177 to inhibit calcium crossing the cell membrane of xenopus oocytes transfected with the alpha 1H subunit of a calcium channel. Legend A is a graph when 10μM TH-1177 was added to the medium, B is a graph of the effect on the washout of TH-1177 and C is the control.

Figure 42 graphically shows the ability of TH-1177 to inhibit calcium crossing the cell membrane of xenopus oocytes transfected with the alpha 1G subunit of a calcium channel. Point 1 signifies the time when 10μM TH-1177 was added to the medium and Point 2 is a time when washout of TH-1177 occurs.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

In the formula hereinabove, the term refers to a cyclic ring containing 5-10 ring atoms and up to a total of 9 carbon atoms. The cyclic ring may be monocyclic or bicyclic, although it is preferred that it is monocyclic. The cyclic ring may contain up to 3 heteroatoms with the remaining ring atoms being carbon atoms. As used herein, the term heteroatom is O, S or N. Thus, the heterocyclic ring may contain 1-3 nitrogen atoms or one nitrogen atom and 1 or 2 sulfur or oxygen atoms. However, it is preferred that the heterocyclic ring contains 1 or 2 heteroatoms. Besides the nitrogen heteroatom, if a second or third ring heteroatom is present, it is preferred that it is either an oxygen ring atom or a nitrogen ring atom. It is especially preferred that the cyclic ring contains 1 heteroatom, i.e., X is N. It is preferred that the cyclic ring contains a total of 5 or 6 ring atoms and that it is monocyclic.

The cyclic ring is preferably saturated, although it may be partially unsaturated, i.e., it may

contain one or two carbon double bonds. However, in a preferred embodiment, it does not contain any double bonds between the X atom and the adjacent ring atom. Preferred heterocyclic moieties include imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, morpholinyl and the like. Preferred heterocyclic rings are imidazolidinyl, piperidyl, piperazinyl and morpholinyl.

In the formula hereinabove, various substituents identified as R_1, R_{11}, R_2, R_{10}, and R_6 may be attached to the ring atom. As defined herein, the R_1 substituent is attached to the X ring atom; the other substituents are attached to the carbon atoms in the ring.

As used herein, the term "lower alkyl", refers to alkyl groups containing 1-6 carbon atoms, which may be straight-chained or branched. These groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertiary butyl, sec-butyl, aryl pentyl, isopentyl, hexyl, and the like. It is preferred that alkyl contains 1-4 carbon atoms. The most preferred alkyl group is methyl.

The terms "electron withdrawing groups" and "electron donating groups" refer to the ability of a substituent to withdraw or donate electrons, respectively, relative to that of hydrogen if the hydrogen atom occupied the same position in the molecule. These terms are well understood by one skilled in the art and are discussed in Advanced Organic Chemistry, by J. March, 4th ed. John Wiley & Sons, New York, NY pp. 16-18 (1992), and the discussion therein is incorporated by reference. Examples of electron withdrawing groups include halo, especially fluoro, bromo, chloro, iodo and the like, nitro, nitrile and the like. Examples of electron donating groups include such groups as hydroxy;

-16-

lower alkoxy, including methoxy, ethoxy and the like; lower alkyl; amino; lower alkylamino; diloweralkylamino; and the like. One skilled in the art will appreciate that the aforesaid substituents may have electron donating properties under one set of circumstances and electron withdrawing properties under different chemical conditions or circumstances; these are also contemplated to be within the scope of these terms. Moreover, the present invention contemplates any combination of substituents selected from the above-identified terms.

As defined herein, the cyclic structure must contain either a Q or Q' substituent, as defined herein, or it may contain both Q and Q'.

Q and Q' may be the same or different and are defined as

$$(CH_2)n_1 - Y - (CH_2)n_2 - CH \overset{\diagup R_3}{\diagdown} R_4.$$
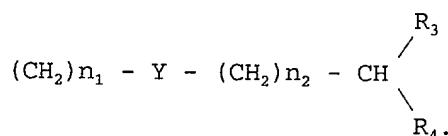
wherein n_1, Y, n_2, R_3 and R_4 are as defined herein. It is preferred that n_2 is 0. Preferred values of n_1 are 1-4, but especially 1. It is also preferred that Y is CH_2 or O, especially O. The preferred values of R_3 and R_4 are independently aryl groups, which may be unsubstituted or substituted. The preferred aryl group is phenyl. It is preferred that the phenyl group be unsubstituted or substituted with halo, especially F, Cl or Br; lower alkoxy, e.g., methoxy; amino; nitro; nitrilo or lower alkyl.

R_1 as defined hereinabove has the formula:

$(CH_2)n - Z - R_5$, wherein n, Z and R_5 are as defined hereinabove.

The preferred value of Z is CH_2 or O.

-17-

It is preferred that n is 1-4, but especially, 1, 2 or 3.

The preferred R_5 is an aromatic ring, especially phenyl, which is either unsubstituted or substituted. If substituted, it is preferred that it is substituted with halo, alkoxy, alkyl, nitrilo, nitro or amino.

In one preferred embodiment of the present invention, the compound of the present invention has the formula:

$$\text{structure IA: pyrrolidine ring with } N\text{-}R_1 \text{ and 2-}R_2 \text{ substituents}$$

IA

It is even more preferred that the compound of Formula IA has the formula:

$$\text{structure: pyrrolidine } N\text{-}R_1, \text{ 2-(CH}_2)_{n_1}\text{-O-CH with two phenyl rings bearing } (R_7)_{n_4} \text{ and } (R_8)_{n_3}$$

wherein

R_1 and n_1 are as defined hereinabove and

R_7 and R_8 are independently hydrogen, or an electron donating group or electron withdrawing group and n_4 and n_3 are independently 1-5.

Another preferred embodiment of the present invention is directed to compounds of the formula:

$$\text{structure IB: piperidine ring with 2-}R_2 \text{ and } N\text{-}R_1$$

IB

-18-

wherein

R$_1$ and R$_2$ are as defined hereinabove.

Even more preferred embodiments of the compounds of Formula IB have the formulae:

and
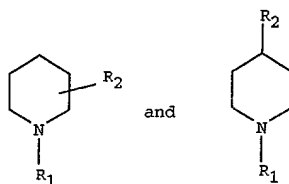
wherein

R$_1$ and R$_2$ are as defined hereinabove.

Especially preferred embodiments of the compounds of Formula IB have the formula:

and

-19-
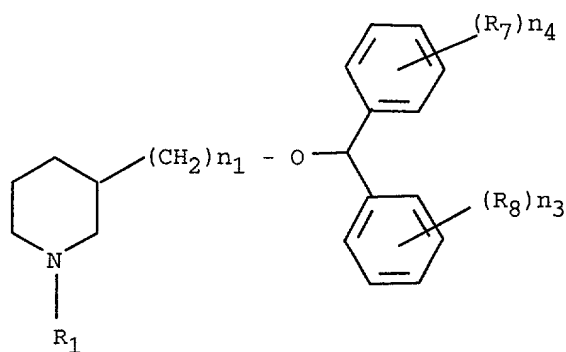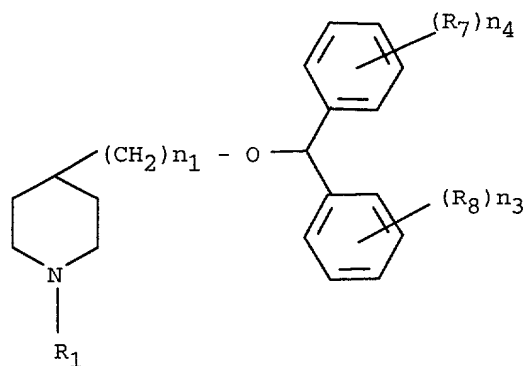
wherein

R_7, R_8, R_1, n_1, n_4 and n_3 are as defined herein.

In the above formula, it is preferred that R_1 has the formula:

$$-(CH_2)_n-\left\langle\!\!\!\left\langle\right.\right.\!\!\!\right\rangle-(R_9)n_5$$
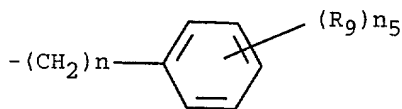
wherein

R_9 is hydrogen, electron withdrawing group or electron donating group and n_5 is 1-5.

Another preferred embodiment has the formula:

$$\overset{R_2}{\underset{R_1}{\bigcirc}}$$
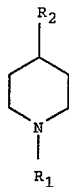
wherein

R_1 is Q and R_2 is hydrogen or Q', as defined herein.

It is preferred that the compounds of the present invention be substantially pure, i.e., substantially free from impurities. It is most preferred that the compounds of the present invention be at least 75% pure (w/w) and more preferably greater than 90% pure (w/w) and most preferably greater than about 95% pure (w/w).

It is also preferred that the compounds of the present invention are enantiomerically pure, i.e., present in substantially one isomeric form, e.g., substantially the R (or D) stereoisomer or the corresponding S (or L) stereoisomer around the asymmetric

-20-

carbon to which is attached the R_2 substituent. It is preferred that the stereochemistry at this carbon site is in the S (or L) configuration.

It is to be understood that all combinations and permutations of the various Markush groups for the different variables are contemplated to be within the scope of the present invention. In addition, the various stereoisomers generated therefrom are also contemplated to be within the scope of the present invention.

Preferred compounds of the present invention are listed on Page 14 herein.

The compounds of the present invention are prepared by art recognized techniques from commercially available starting materials. Exemplary procedures for making the compounds of the present invention are outlined hereinbelow.

For example, a compound of the formula II:
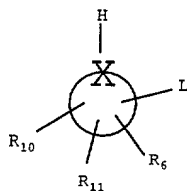
wherein

X is nitrogen reacts with R_{20} COOH or an acylating derivative (e.g., lower alkyl ester) thereof under amide forming conditions to form the corresponding amide:
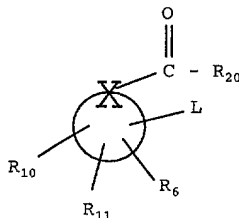
wherein

R_1, R_{10}, R_{11} and R_6 are as defined hereinabove

R_{20} is $(CH_2)_{n-1}-Z\ R_5$, $CH_2)_{n-1}-Y-(CH_2)_{n2}-CH\overset{\displaystyle R_3}{\underset{\displaystyle R_4}{\Big\langle}}$,
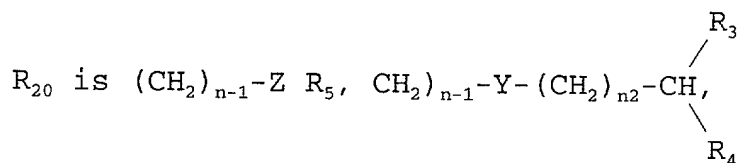
H, or lower alkyl, containing one less CH_2 group than the alkyl group of R_1, and

L is a good leaving group, such as tosylate, mesylate, halide (e.g., chloride, bromide or iodide), and the like.

The product thereof is reacted with a carbonyl reducing agent, such as lithium aluminum hydride, in the presence of a Lewis acid, e.g., $AlCl_3$ to form the corresponding alkane. The product thereof is reacted with R_2L_1, under substitution reaction conditions, wherein R_2 is as defined hereinabove and L_1, is a good leaving group, e.g. halide, tosylate or mesylate to form the product of the present invention.

When Y in R_2 is O, and X is NH then the product is formed by a variation of the procedure described hereinabove. For example,
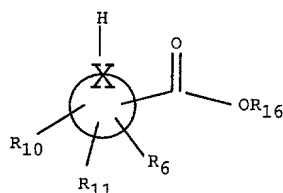
is reacted with

R_{20} COOH or acylating derivative under amide forming conditions to form a product of the formula:

-22-
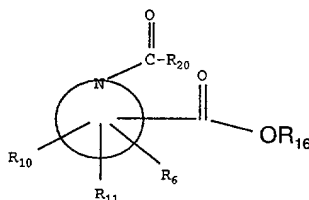
wherein R_{16} is lower alkyl and R_{20} is as defined
hereinabove. When the above product is reacted with a
reducing agent, such as lithium aluminum hydride, in the
presence of a Lewis acid, such as $AlCl_3$, not only does
this reagent reduce the corresponding amide but it also
reduces the ester functionality to form the following
compound:
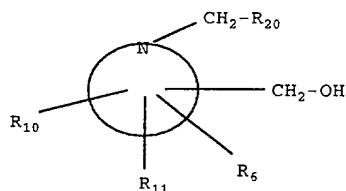
The corresponding alcohol is reacted with a molecule of
the formula:

$$L-(CH)n_2 - CH \begin{array}{c} R_3 \\ \diagup \\ \diagdown \\ R_4 \end{array}$$
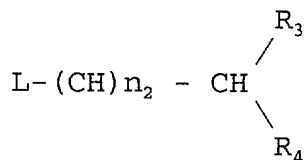
wherein L is a good leaving group, such as halide,
tosylate, mesylate or the like in the presence of a
strong base, such as hydroxide or when L is OH, in the
presence of a catalytic acid e.g., paratoluenesulfonic
base under Williamson reaction conditions to form the
compounds of the present invention.

It is to be noted that in both syntheses R_1 is
defined to include CH_2-R_{20}.

However, if R_1 is H, then the compound of
Formula II is heated with R_2L_1 under nucleophilic reaction
conditions, as described hereinabove, to form the product
of Formula I.

-23-

If X is carbon, then the compound of Formula I may be formed by reacting a carbonyl compound of Formula III,

5

$$
\begin{array}{c}
O \\
\parallel \\
C \\
R_1 - - L \\
R_{10} R_6 \\
R_{11}
\end{array}
\qquad III
$$
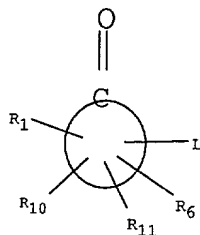
10   with a Grignard reagent R_1MgX_1 under Grignard reaction conditions to form the corresponding alcohol of Formula IV,
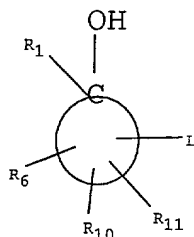
$$
\begin{array}{c}
OH \\
R_1 \,| \\
C \\
 - L \\
R_6 \\
R_{10} R_{11}
\end{array}
\qquad IV
$$

wherein R_1, R_{10}, R_{11}, R_6 and L are as defined hereinabove
20   and X_1 is halide. It is to be noted that the carbonyl compound of Formula III is prepared from the corresponding alcohol by oxidizing the alcohol with an oxidizing agent known in the prior art such as $KMNO_4$, CrO_3, $K_2Cr_2O_7$ and the like under oxidizing conditions.

25   The OH functionality in Formula IV is converted to CH by techniques known in the art, such as, for example, by converting the alcohol to a tosylate or other sulfonates (e.g., mesylate) and then reacting the product thereof with a reducing agent such as $LiAlH_4$ or $NaBH_4$ and
30   the like in a dipolar aprotic solvent, e.g., Et_2O, to form the corresponding alkane.

-24-

$$\begin{array}{c} H \\ R_1 \diagdown \; | \\ C \\ \bigcirc \!\!\!\!\!\!-\!\! L \\ R_6 \diagup \; | \diagdown \\ R_{10} \quad R_{11} \end{array}$$
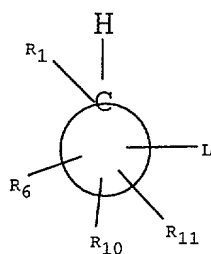
Then the product is reacted with R_2L, under substitution reaction conditions as described hereinabove to form the compound of the present invention.

If groups are present on the substituents R_1, R_2, R_6, R_{10}, or R_{11} that are reactive with the reagents used, then prior to the reaction, they may be protected by reacting them with protecting groups known in the art. Examples of protecting groups are described in the book entitled "Protective Groups in Organic Synthesis", by Theodora W. Greene, John Wiley & Sons, New York, NY 1981, the contents of which are incorporated by reference.

In the reactions described hereinabove, it is preferred that the reactions be conducted in solvents which are not reactive with the reactants or the products. In the amide forming reactions, it is preferred that the reaction be conducted in such solvents as methylene chloride, chloroform, and the like, while in the Grignard reactions and the reduction reactions, it is preferred that the reaction be conducted in ethers, such as diethyl ether, tetrahydrofuran and the like. The substitution and Williamson reactions are preferably conducted in inert solvents, such as hexane, pentanes, hexane, toluene, petroleum ether and the like. The reactions are conducted at temperatures effective to form the desired products in each step. Preferably these

-25-

temperatures range from about 0°C to refluxing temperatures of the solvent, depending on the particular reaction. A skilled artisan can easily determine the reaction conditions. However, for the Grignard reaction it is preferred that the reaction be conducted at near freezing temperatures (e.g., 0°C or less), while in the substitution and the Williamson reactions, it is preferred that the reaction be conducted at refluxing temperatures.

The examples described hereinbelow provide exemplary procedures for preparing compounds of the present invention using the schematics described hereinabove.

The compounds of the present invention exhibit excellent cytostatic activity when administered in amounts ranging from about 0.5 mg to about 100 mg per kilogram of body weight per day. A preferred dosage regimen ranges from about 1 mg per kilogram per day to about 50 mg per kilogram per day. This dosage regime may be adjusted by the physician to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A decided practical advantage is that the active compound may be administered in an convenient manner such as by the oral, intravenous (where water- soluble), intramuscular or subcutaneous routes.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatine capsules, or it may be compressed into tablets, or it may be incorporated directly into the food

-26-

of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 3 and 1000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of

-27-

course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations. For example, sustained release dosage forms are contemplated wherein the active ingredient is bound to an ion exchange resin which, optionally, can be coated with a diffusion barrier coating to modify the release properties of the resin.

The active compound may also be administered parenterally or intraperitioneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixture thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions

-28-

and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic

-29-

compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parental compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specifics for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased conditions in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore described. A unit dosage form can, for example, contain the principal active compound in amounts ranging from about 3 to about 1000 mg. Expressed in proportions, the active compound is generally present in from about 1 to about 750 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

Unless indicated to the contrary, percentages are by weight.

For a better understanding of the present invention reference is made to the following non-limiting description and examples.

GENERAL METHODS

A.   Synthesis of TH-1177.   TH-1177 was synthesized in three simple steps (Figure 1), Step 1 forms the amide, Step 2 is the reduction of the amide and Step 3 is the ether formation under Williamson ether formations.

Step 1:   L-proline methyl ester was coupled with 4-methoxyphenylacetic acid using Benzotriazol-1-yl-oxytripyrrolidinephosphonium (PyBOP) and 2 equivalents of N-methylmorpholine to generate methyl 1-[2-(4-methoxyphenyl)acetyl]pyrrolidine-2-carboxylate, a yellowish oil.

Step 2:   The resulting amide was subsequently reduced to the amino alcohol with $LiAlH_4$ and $AlCl_3$ in THF.

Step 3:   The colorless oil was converted to its amine alcohol salt by dissolving the product of Step 2 in a small amount of ethyl acetate and adding 15% HCl/ethyl acetate solution and evaporating the solvent using a rotary vacuum.   The salt was coupled with 4-chlorobenzhydrol under Williamson conditions with catalytic para-toluene sulfonic acid in refluxing toluene.   The final brownish oil was isolated by column chromatography on silica gel using a 50:50 mixture of ethyl acetate and hexane and confirmed by NMR and mass

spectrometry. TH-1177 was dissolved in DMSO for use *in vitro* in ethanol for use *in vivo*.

With the exception of the last coupling, all steps provide yields of greater than 75%. PyBOP provides a safe and highly dependable method of forming amides by coupling a variety of amines and acids. It has proven superior to formation of acid halides or to the use of other coupling agents such as dicyclohexylcarbodiimide. Efficient reduction of both the amide and the ester is achieved in one step using $LiAlH_4$ and $AlCl_3$ in a 1:3 ratio. For the purity of the final product, it is important to have sufficient amount of hydride present to avoid formation of the aldehyde. Three different syntheses of TH-1177 were used for the completion of these studies, with no differences in NMR or mass spectroscopy characteristics among the batches. Each batch was assessed for its ability to inhibit PC3 and LNCaP prostate cancer cell proliferation *in vitro* (see Example 4) and the IC50 values for each batch were within the variance of the assay.

The three-step synthesis of TH-1177 (Figure 1) provides the sample outline of the synthesis that allows for large number of targets to be made easily and efficiently.

The other compounds listed hereinbelow are prepared by a variation of the above procedure. In all cases, Step 2 and Step 3 are very similar.

B.   Synthesis of TH-1087

Step 1: Ethyl isonipecotate (1 equivalent) and 4-methoxyphenylacetic acid (1.2 equivalents) was reacted with 1 equivalent of benzotriazol-1-yl-oxytripyrrolidine

-32-

phosphonium (PyBOP), and 1.2 equivalents of N-methyl morpholine (NMM) in methylene chloride and stirred at room temperature under an inert gas, such as nitrogen, for 30 minutes to an hour. The solvent was evaporated off.

Step 2: The product of Step 1 (1 equivalent) was reacted with 1 equivalent of LiAlH$_4$ and 1/3 equivalent of AlCl$_3$ (relative to LiAlH$_4$). More specifically, to a round bottom flask equipped with stir bar and dry THF, AlCl$_3$ and LiAlH$_4$ were carefully added. The resulting solution was then allowed to stir for about 1 hour. The amide product of Step 1 was dissolved in a minimal amount of solvent (THF) and added to the stirring solution via a syringe very slowly to form the alcohol. After completion of the reaction, the THF was evaporated.

Step 3: The product of Step 2 was converted to the amino alcohol salt from the amino alcohol by dissolving the product of Step 2 in a small amount of ethyl acetate. 15% of HCl/ethyl acetate solution was added thereto. The salt was isolated by evaporating the solvent by rotary vacuum. To a round bottom flask equipped with stir bar and solvent the salt (1 equivalent) was added. To this was added 0.5 equivalents of p-TsOH and 1.1 equivalent of benzhydrol and the mixture was refluxed until completion. The product was isolated by column chromatography on silica gel using a 50:50 mixture of ethyl acetate and hexane.

C. TH-2029. The procedure in Example B was followed except that in Step 3, 4-chlorobenzhydrol was utilized.

D. TH-2043. The procedure in Example B was followed, except that in Step 3, 4,4'-dichlorobenzhydrol was utilized.

        E. TH-1203. The procedure in Example B was followed except in Step 1, ethyl nipecotate was utilized.

        F. TH-1205. The procedure in Example C was followed, except in Step 3, 4-chlorobenzyhydrol was utilized.

        G. TH-1019. The procedure in Example B was followed, except in Step 1, 3-(2-methoxyphenyl) propionic acid was utilized.

        H. MMR-64. To a round bottom flask equipped with stir bar containing toluene; 5 equivalents of diphenylacetic acid was added followed by 5 equivalents of DMF. The solution was allowed to stir at 0°C until the temperature had equilibrated. Upon reaching 0°C, the oxalyl chloride was added slowly, and the solution was allowed to warm to room temperature. It was stirred at room temperature for about 1 hour, upon which it was placed back onto ice (0°C) and piperidine (1 equivalent) was slowly added. The reaction was allowed to warm to room temperature and was stirred until completion. The toluene was removed by rotary evaporation and the amide was isolated.

        To a round bottom flask equipped with stir bar and dry THF, 1 equivalent of $LiAPH_4$ and 1/3 equivalents of $AlCl_3$ (relative to $LiAlH_4$) were added slowly. The resulting solution was allowed to stir for about 1 hour.

-34-

The amide formed hereinabove (1 equivalent) was dissolved
in a minimal amount of THF and added to the stirring
solution via syringe very slowly.

I. MMR-70.

Step 1: To a round bottom flask equipped with
stir bar and toluene, 5 equivalents of diphenylacetic
acid was added, followed by 5 equivalents of DMF. This
solution was allowed to stir at 0°C until the temperature
had equilibrated. Upon reaching 0°C, the oxalyl chloride
was added slowly and the solution was allowed to warm to
room temperature. It was stirred at room temperature for
about 1 hour, at which time it was placed back onto ice
and ethyl isonipecotate (1 equivalent) was added slowly.
After the addition of amine, the solution was then
allowed to warm to room temperature as the reaction was
stirred until completion.

Step 2: The procedure of Step 2 of B was
followed.

Step 3: The procedure of Step 3 of B was
followed.

J. MMR-92. The procedure of Example I was
followed except in Step 3, 4-chlorobenzhydrol was
utilized.

K. MMR-100. The procedure of Example I was
followed, except in Step 3, 4,4'-dichlorobenzhydrol was
utilized.

L. MMR-104. The procedure of Example I was
followed, except 9-fluorenol was utilized in Step 3.

-35-

M. TH-1113. The procedure of TH-1177 was followed, except that benzhydrol was utilized in Step 3.

N. TH-1211. The procedure of TH-1177 was followed, except that the D isomer of Proline Methyl ester hydrochloride was utilized in Step 1.

O. TH-2019. The procedure of TH-1177 was followed except that in Step 3, 4,4'-dichlorobenzhydrol was utilized.

P. TH-2129. The procedure of TH-1177 was followed, except that in Step 3, 4,4'dimethoxybenzhydrol was utilized.

Q. TH-2083. The procedure of TH-1177 was followed, except that 3-(4-methoxyphenyl) propionic acid was utilized in Step 1 and benzhydrol was utilized in Step 3.

R. TH-2085. The procedure of TH-2083 was followed except that in Step 3, 9-fluorenol was utilized.

S. TH-2151. The procedure for the synthesis of TH-2083 was followed except in Step 1, 3-(2-methoxyphenyl) propionic acid was utilized and in Step 3, 4-chlorobenzhydrol was utilized.

T. TH-2153. The procedure for the synthesis of TH-2151 was followed except in Step 3, 4,4'-dimethoxybenzhydrol was utilized.

U. TH-2209. The procedure for the synthesis of TH-1177 was followed except in Step 3, 4-bromobenzhydrol was utilized.

5

V. TH-2149. 2-(1-methylpyrrolidin-2-yl)-ethan-1-ol was dissolved in a small amount of ethyl acetate. To this was added a 15% HCl/ethyl acetate solution. The ethyl acetate was removed by rotary vacuum, thereby forming the amine salt. To this salt (1

10 equivalent) was added toluene, 0.5 equivalents paratoluenesulfonic acid and 1.1 equivalents of benzhydrol. The reaction was refluxed until completion.

W. TH 3101. The procedure for the synthesis

15 of TH-1177 was followed except in Step 1, 4-methoxyphenylacetic acid was replaced with 4-fluorophenylacetic acid.

X. TH 3104. The procedure for the synthesis

20 of TH-1177 was followed except 2-methoxyphenylacetic acid was used instead of 4-methoxyphenylacetic acid.

Y. TH 3105. The procedure for the synthesis of TH-1177 was followed except 3-methoxyphenylacetic acid

25 was used instead of 4-methoxyphenylacetic acid.

The various products identified in A-Y are listed hereinbelow in Table 1:

-37-

TABLE 1
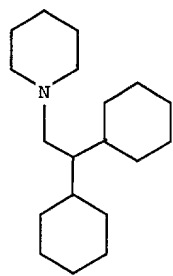
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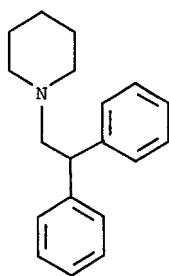
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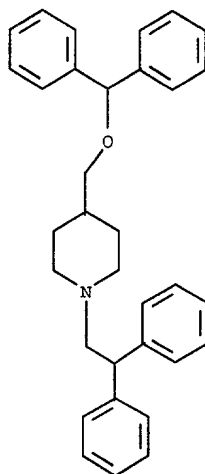
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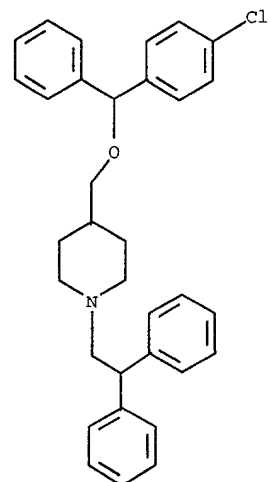
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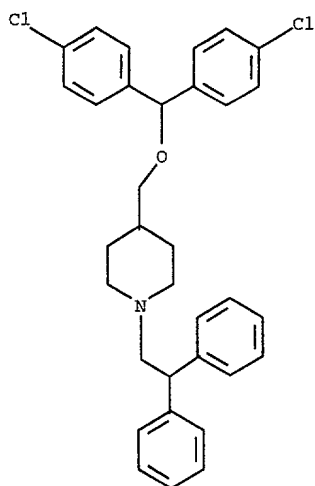
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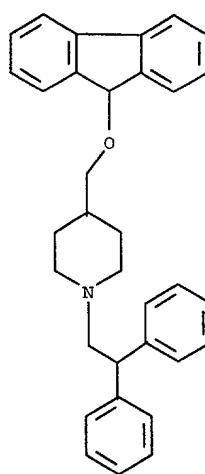
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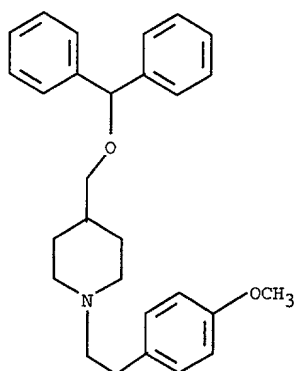
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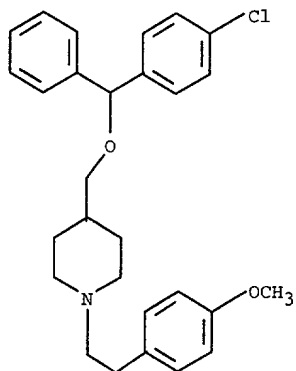
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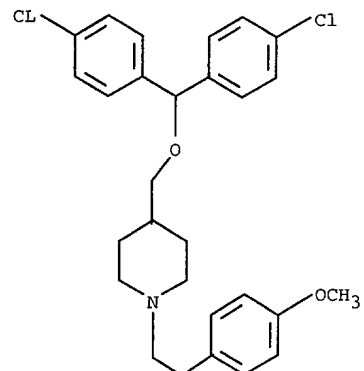
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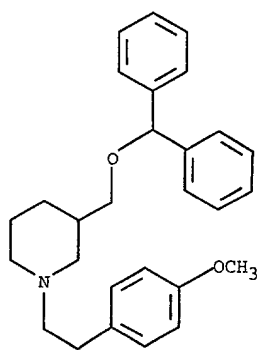
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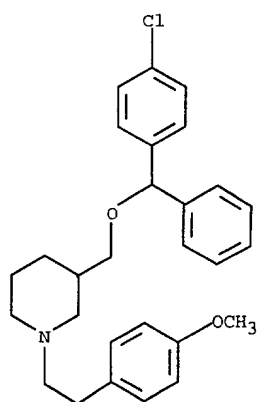
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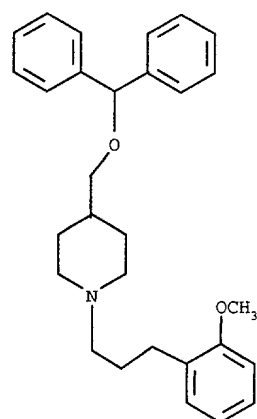
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TH-2149

TH-1113

TH-1177

-39-
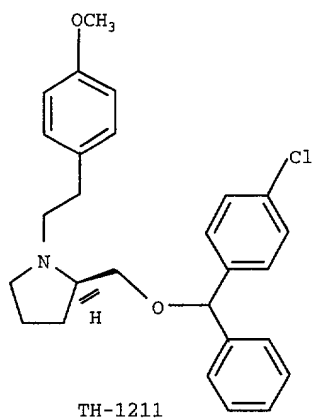
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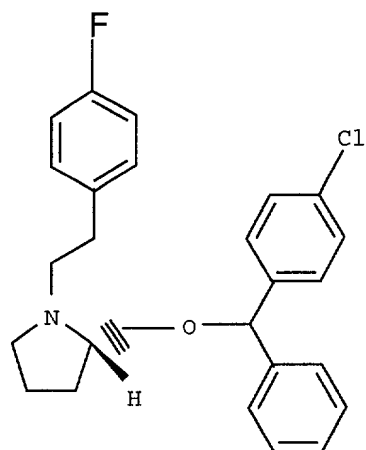
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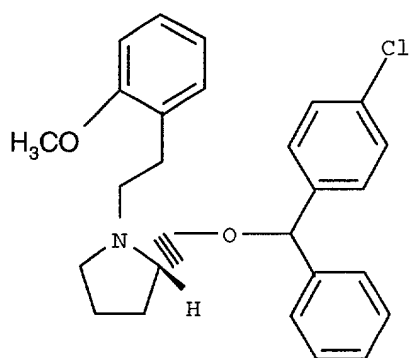
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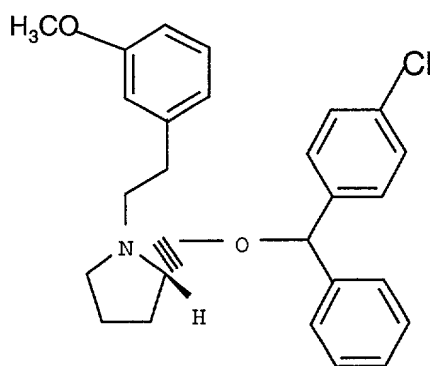TH-2083

TH-2085

TH-2151

TH-2153

TH-2209

-40-
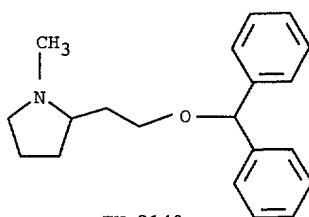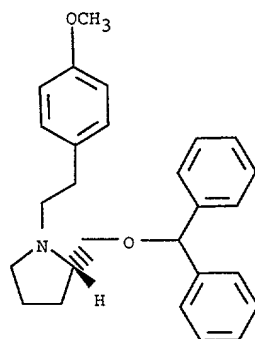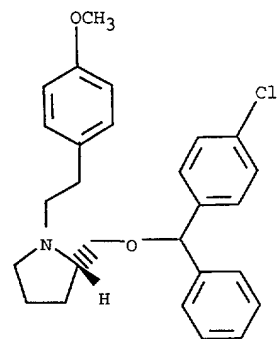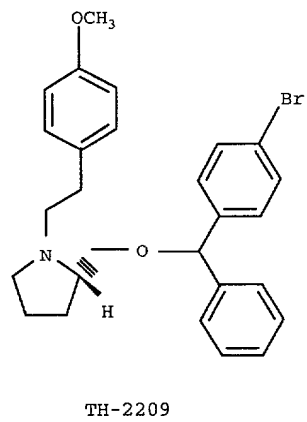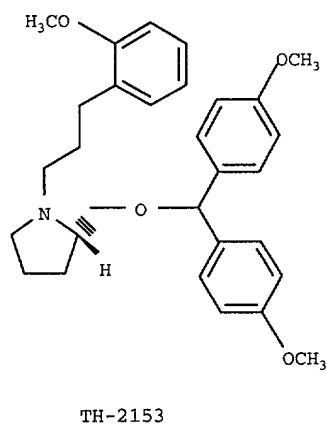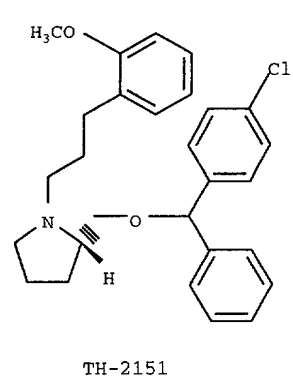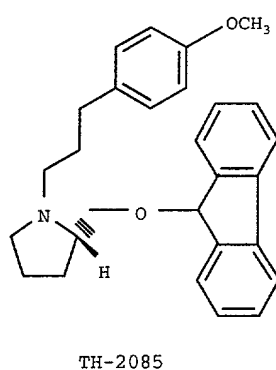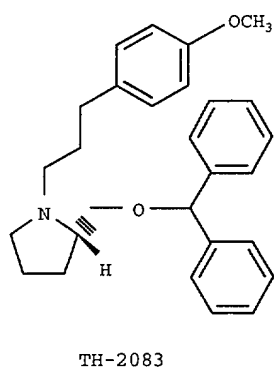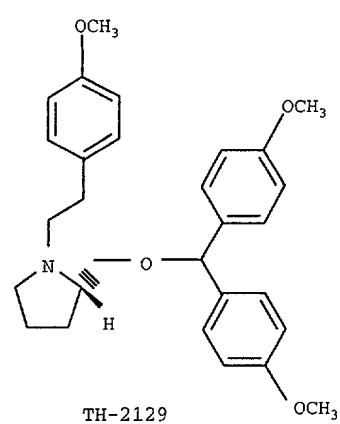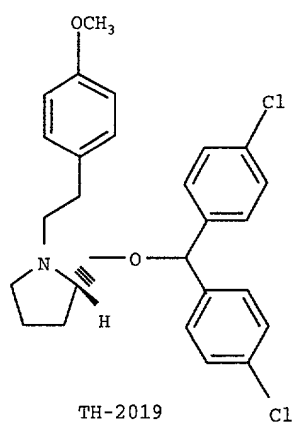
TH-3101

TH-3104

TH-3105

The compounds of the present invention are useful as anti-tumor agents. For example, compounds of the present invention are effective in treating malignant tumors, such as leukemia, especially lymphatic leukemia and solid tumors, for example, breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, carcinomas, (e.g., adenocarcinomas,) melanomas, lymphomas, sarcomas (such as osteosarcomas), malignacies or tumors arising from tissue in lung, colon, liver, reproductive organs, (e.g., testes, uterus), skin, bone and connective tissue, central nervous system (CNS), e.g., brain, and peripheral nerve, including glia and Schwann cells, and the like.

Without wishing to be bound, it is believed that the compounds of the present invention are calcium channel blockers.

It is believed that an increase in intracellular concentration of calcium is provided in response to stimulation, such as by mitogens. As used herein, the term mitogen is an agent that causes cells to divide and multiply, i.e., a stimulant of mitosis. Examples of mitogens include growth stimulating factors, such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), bradykinin, platelet derived growth factor (PDGF), and the like. Without wishing to be bound, it is believed that the growth stimulating factor engages with its receptor on the cell, which results through a cascade of reactions, in the production of inositol triphosphate (IP-3). Moreover, without wishing to be bound, it is believed that IP-3, binding to a specific intracellular receptor, induces the release of calcium from an intracellular storage pool, such as the

endoplasmic reticulum, which in turn triggers influx of extracellular calcium into the cell.

It is believed that calcium entry is a critical signal for numerous cell processes, especially cellular activation and proliferation. The entry is controlled by membrane spanning pores known collectively as calcium channels. Opening of calcium channels allows calcium to follow its electrochemical gradient into the cytosol. These channels are classified by biophysical properties, such as conductance and mean open time and by relative sensitivity to various pharmacological agents.

There are various types of calcium channels that are known, such as L, N, P, Q, R, S and T. However, without wishing to be bound, it is believed that cancer cells possess calcium channels that have the properties similar to calcium channels in the T-family. Moreover, without wishing to be bound, it is also believed that cell proliferation associated with cancer is activated by calcium entry into the cytosol or cell interior through the calcium T-like channel.

As used herein the term T-like channel refers to a calcium channel that has the characteristics of a T-channel. However, unlike a true T-channel, the T-like channels are stimulated by a second messenger, such as calmodulin.

It is furthermore believed that the compounds of the present invention are primarily T-like calcium channel antagonists, that is, they retard and/or prevent the passage of calcium through the calcium T-like channels and entry thereof into the cell and as a result, are effective in retarding cellular proliferation. More

specifically, it is believed that T-like calcium channels are found in electrically non-excitable cells, where calcium entry is believed to be conducted by non-voltage gated (NVG) channels. It is believed, without wishing to be bound, that cancer is associated with the entry of calcium through these type of channels to the cytosol and that the compounds of the present invention inhibit the passage of calcium through these types of T-channels, which conduct calcium entry by non-voltage gated channels, and thereby the compounds of the present invention inhibit unregulated proliferation of cancer cells.

Electrically non-excitable cells as used herein are any cells which are not electrically excitable, that is, cells which do not exhibit action potentials, such as occurs in neurons and muscle cells. In these cells, the calcium influx is not initiated by electrical action potential response at the plasma membrane. Electrically non-excitable cells contain the calcium T-like receptor operated calcium channels.

The compounds of the present invention are unlike most chemotherapeutic drugs. Most conventional cancer chemotherapeutic drugs are cytotoxic and exert their therapeutic benefit by killing cancer cells. On the other hand, the compounds of the present invention act by inhibition of calcium entry and arrest or retard cell proliferation controlling the growth of cancer cells and in this way renders the disease effectively impotent.

The present inventors have found that compounds which inhibit calcium entry and/or inhibit cellular proliferation in non-excitable cells are useful in treating cancer. Treating, as used herein means

-44-

ameliorating a disease such that the condition of the patient improves or such that the progress of the disease is slowed.

Thus another embodiment of the present invention is directed to a method of treating cancer in an animal afflicted with such disease which comprises administering to said animal a calcium blocker that prevents and/or retards the entry of calcium ions across the cell membrane in the cancer cells in response to a mitogenic stimulus. The preferred calcium blocker is an organic compound that contains at least one carbon-carbon bond. It is therefore, preferably not an inorganic compound. The calcium blocker is present in amounts effective to retard the passage of extracellular calcium ions into the cells. The preferred effective amounts of the calcium blocker present are described hereinabove. The preferred calcium blocker contains a heterocyclic ring containing at least one nitrogen ring heteroatom. It is even more preferred that the cyclic ring contains 5-10 ring atoms and 1-3 heteroatoms, as long as at least one of the ring heteroatoms is nitrogen. It is especially preferred that the heterocyclic ring is saturated.

In a preferred embodiment, the calcium blocker inhibits or retards the entry of calcium ions to the cancer cell by blocking a calcium channel, such as the T-like calcium channel. Without wishing to be bound, it is believed that the calcium channel blocker inhibits or retards calcium entry into the cell by interacting with $\alpha 1$ subunits such as $\alpha 1G$ or $\alpha 1H$ subunits of the T-like calcium channel. The preferred calcium blockers are compounds of Formula I described hereinabove.

-45-

The effectiveness of various test compounds can be monitored by such tests, as described hereinbelow, which measure the calcium antagonist activity utilizing non-excitable cells or by monitoring the decrease in
5   proliferation of cancer cells.

Suitable electrically non-excitable cells useful in accordance with these assays include any cell which requires the entry of calcium, for activation or proliferation, but which do not initiate entry of calcium
10   by an electrical action potential such as occurs in neurons. Non-excitable cells include lymphocytes, and other formed elements of the blood epithelial cells, connective tissue cells and secretory cells including glandular cells. Particularly preferred electrically
15   non-excitable cells for use in vivo assays include Jurkat cells (T-lymphocyte), MDA-468 (a breast cancer cell line), PC-3 (a prostate cell line) A-549 (a lung cancer cell line), HCT-116 (a colon cancer cell line), SK-OV3 (an ovarian cancer cell line), MIA
20   PaCa-2, (a pancreatic cell line), and any other cell type or cell line which is electrically non-excitable. The cell lines mentioned above are available from the American Type Culture Collection, Manassas, VA.

The cell lines used in the assays described
25   hereinbelow were maintained and remained viable using standard techniques known in the art. For example, for hormone-resistant LNCaP-FRG and hormone sensitive PC3 prostate cancer, cells which were obtained from the ATCC (Manassas, VA), were maintained in RPMI 1640,
30   supplemented with glutamine and 5% fetal bovine serum containing SerXtend (Irvine Scientific). The fetal

-46-

bovine serum used for culture was heat inactivated by maintaining the serum at 56°C for 1 hr.

Although various assays are known in the art, the present inventors have utilized the following assays.

Measurement of intracellular Ca^{2+} concentration: Cells were incubated in growth media containing 1µM of the acetoxy-methyl ester of the Ca^{2+} -sensitive fluorescent dye indo-1 (indo-1/AM, Molecular Probes, Eugene, OR) for 1 hour at 37°C. Cells were washed three times in buffer A (10mM HEPES, pH 7.4, 1 mM MgCl$_2$, 3mM KCl, 1mM CaCl$_2$, 140 mM NaCl, 0.1% glucose, 1% fetal bovine serum) and suspended to a final concentration of 10^6/ml. Prior to stimulation, cells were warmed to 37°C. Prior to stimulation, cells were also incubated with drug, calcium channel antagonists and the IC$_{50}$ values were determined. Changes in [Ca^{2+}] were monitored in an SLM 8100C spectrofluorometer (SLM/Aminco, Urbana, IL) using previously published methods, Haverstick, et al. (1998) Cell Calcium, 23:361-368; Densmore, et al. (1996) Am. J. Physiol., 271:C1494-1503, incorporated herein by reference.

Calcium influx into the cell is suitably stimulated using either (1) a physiological ligand or (2) an endoplasmic reticulum (ER) ATPase inhibitor. Preferably, a physiological ligand is used to stimulate calcium influx into the cell.

A physiological ligand as used herein is a ligand which binds to a receptor on a non-electrically excitable cell and stimulates Ca^{2+} influx into the cell. Depending on the non-electrically excitable cell used, these ligands will vary. For example, suitable ligands for use with Jurkat cells include antibodies to the T-

-47-

like cell receptor for antigen, e.g., OKT3. Suitable
ligands for use with MDA 468 cells include epidermal
growth factor or transforming growth factor. Suitable
ligands for use with PC-3 cells include epidermal growth

5   factor or purinergic agonists such as adenosine. These
are reviewed in Carpenter and Cohen, 1990, J. Biol. Chem.
265: 7709-7712; Crabtree and Clipstone, 1994. Annu. Rev.
Biochem. 63:1045-1083; and Gardner, P. 1989. Cell,
59:15-20 incorporated herein by reference. These

10   reagents are available from several suppliers such as
Sigma, CALBIOCHEM and Research Diagnostics (Flanders,
NY).

Endoplasmic Reticulum (ER) ATPase inhibitor, as
used herein, is any compound which stimulates the release

15   of calcium from the endoplasmic reticulum into the
cytoplasm, which, in turn, activates calmodulin, which,
in turn, activates calcium entry into the cell (for
review of ER ATPase inhibitors, see Thastrup, O, Agents
and Actions (1990), 29:8-15; Inesi and Sagara, Archives

20   of Biochem. and Biophys., 298: 313-7 and Darby, et al.,
Biological Signals (1993), 2:293-304). Preferred ER
ATPase inhibitors include cyclopiazonic acid (available
from Sigma, St. Louis, MO) and thapsigargin (available
from Sigma).

25   **Measurement of cellular proliferation:** LNCaP
cells at 2.5×10^4/well or PC3 cells at 5×10^4/well, both
in a final volume of 100 μl, were plated in triplicate in
standard flat bottom 96 well tissue culture plates in the
presence of drug or vehicle (DMSO). Unless otherwise

30   indicated, cells were grown for 48 hours at 37°C in a CO_2
incubator. Relative cell growth was determined with the
CellTiter 96 aqueous cell proliferation assay (Promega,

-48-

Madison, WI) as described by the manufacturer using an
automated plate reader. Results were calculated in a
blinded fashion and are means of triplicate
determinations.

5   For the 48 hour proliferation, cells were
cultured with calcium channel antagonists or without
calcium channel antagonists. The IC_{50} values of the
calcium channel antagonists were determined. The present
inventors have found that compounds which have an IC_{50}

10   value less than about 10μM and more preferably less than
5μM and most preferably less than 2 μM with respect to
the breast cancer cell lines, prostate cancer cell lines
and lymphocytic leukemia cell lines in either or both of
the above-identified assays are effective in treating

15   cancer in patients, including mammals and especially
humans. In addition, preferred compounds do not possess
an imidazole moiety. The present inventors have found
that compounds of the present invention have efficacy of
this magnitude in these cell lines.

20   For the other cell lines of solid tumors, such
as pancreatic tumor cell lines, ovarian tumor cell lines,
lung tumor cell lines, the present inventors have found
that compounds which exhibit IC_{50} of less than about 30μM
and more preferably less than about 20μM and most

25   preferably less than about 10μM in either or both of the
aforementioned assays are effective in treating cancer in
patients, including mammals, and especially humans.
Again, the inventors have found that compounds of the
present invention exhibit efficacy of this magnitude in

30   these cell lines.

         In many of the experiments described below, in
vivo assays were also performed.

-49-

Animal Studies: All protocols were approved by the Animal Care Committee of the University of Virginia. SCID mice were housed in a barrier isolation facility of the University of Virginia Department of Comparative Medicine and all personnel observed sterile techniques when entering the facility and handling animals. TH-1177 was dissolved in ethanol and diluted 10-fold in sterile phosphate buffered saline (PBS) immediately prior to each day's injection. Injection volumes of 0.5 ml per animal were used. Vehicle consisted of PBS diluted ethanol. PC3 cells for injection were prepared by washing in sterile PBS three times prior to suspension to 2×10^6 per ml. Each animal received 0.5 ml of cells by IP injection on day zero of the experiment shown in Figure 9. Each animal received a daily IP injection of vehicle or drug beginning on day 1.

Statistical methods: *In vivo* survival data were analyzed with Prism 2.01 (GraphPad Software, San Diego, CA). The results of the Kaplan-Meier analysis are presented as one-tailed probabilities because there was no reasonable expectation that drug treatment would cause the mice to succumb more rapidly to the implanted cancer than would control animals.

EXAMPLE 1

TH-1177 blocked capacitative Ca^{2+} entry in human prostate cancer cells. In electrically non-excitable cells, Ca^{2+} influx is triggered by release of Ca^{2+} from its internal storage depot by a phenomenon that has been called "capacitative" Ca^{2+} entry (Putney, J.W., Jr. (1986) *Cell Calcium*, 7:1-12; Haverstick, et al. (1993) *Mol. Biol. Cell*, 4:173-184; Kohn, et al. *Proc. Natl. Acad. Sci. USA*, 92: 1307-1311, incorporated herein by reference). Capacitative entry can be initiated by treatment of cells with thapsigargin. Thapsigargin inhibits the Ca^{2+} -ATPase of the endoplasmic reticulum allowing uncompensated leak of Ca^{2+} from this compartment into the cytosol thereby causing Ca^{2+} entry in the absence of engagement of a specific receptor. (Thastrup, et al. (1990) *Proc. Natl. Acad. Sci. USA*, 87:2466-2470; Takemura, et al. (1989) *J. Biol. Chem.* 264:12266-12271, incorporated herein by reference).

As shown in Figure 2A, addition of TH-1177 to a suspension of LNCaP human prostate cancer cells before thapsigargin resulted in a dose dependent inhibition of the increase in $[Ca^{2+}]_i$. TH-1177 also reduced the increase in $[Ca^{2+}]_i$ initiated by thapsigargin when added after the stimulus (Figure 2B). The elevated $[Ca^{2+}]_i$ seen at 100 s in Figure 2B was attributable to Ca^{2+} entry alone. Therefore, the effect of TH-1177 was mediated by inhibition of Ca^{2+} entry.

EXAMPLE 2
TH-1177 inhibited receptor-linked Ca^{2+} entry.

The P2 purinergic receptor is linked to activation of the Ca^{2+} entry pathway in many types of cells including prostate cancer cells (Fang, et al. (1992) <u>J. Clin. Invest.</u> 89:191-196). The ability of TH-1177 to block capacitative Ca^{2+} entry induced by thapsigargin indicated that this compound block Ca^{2+} entry triggered by the engagement of a specific receptor. The P2 receptor binds extracellular ATP inducing multiple biochemical events including Ca^{2+} entry (Fang, et al. <u>supra</u>). Addition of ATP to LNCaP prostate cancer cells resulted in a rapid rise in [Ca^{2+}]$_i$, that was inhibited by the prior addition of TH-1177 (Figure 3A). As shown in Figure 3B, TH-1177 added after ATP also caused a reduction in the [Ca^{2+}]$_i$ that had been augmented by P2 receptor engagement.

PC3 prostate cancer cells also demonstrated an increase in [Ca^{2+}]$_i$ when stimulated by ATP (Figure 3). In the experiment depicted in Figure 4A, TH-1177 was added before the cells were stimulated with ATP. TH-1177 caused a concentration dependent inhibition of the increase in [Ca^{2+}]$_i$ that was otherwise induced by engagement of the purinergic receptor. By 70 s after ATP addition, release of Ca^{2+} from the internal storage pool was largely over (for example, see Figure 5 below) and the maintenance of elevations of [Ca^{2+}]$_i$ over baseline was dependent on Ca^{2+} entry from the extracellular compartment. As shown in Figure 4B, addition of TH-1177 to cells previously treated with ATP caused a reduction in [Ca^{2+}]$_i$. Therefore, TH-1177 interacts with the Ca^{2+} influx pathway.

-52-

The efficiency of TH-1177 at blocking Ca^{2+} entry was assessed by comparison to the effect of chelation of extracellular Ca^{2+} with EGTA, which was considered 100% inhibition of Ca^{2+} entry. For LNCaP cells, the IC_{50} for

5   TH-1177 was 3μM and for PC3 cells, the IC_{50} was 16μM under these conditions.

The data presented above provide that TH-1177 inhibited the stimulated increase in $[Ca^{2+}]_i$ by blockage of Ca^{2+} entry. In Figure 5, extracellular Ca^{2+} was

10   markedly reduced by addition of EGTA to the extracellular medium. Under these conditions, addition of ATP to either PC3 (Figure 5A) or LNCaP (Figure 5B) cells resulted in a rise in $[Ca^{2+}]_i$ that was more transient and of smaller magnitude than in the presence of

15   extracellular Ca^{2+}. This increase represented release of Ca^{2+} from the internal storage pool. Addition of TH-1177 had no effect on the size of this change in $[Ca^{2+}]_i$. This indicated that TH-1177 did not interfere with Ca^{2+} release and suggested that TH-1177 had no influence on the

20   biochemical events upstream from release of Ca^{2+} from the internal pool. The inhibition of increases in $[Ca^{2+}]_i$ caused by TH-1177 in the presence of extracellular Ca^{2+} was due to blockage of the Ca^{2+} entry pathway that can be opened by engagement of the P2 purinergic receptor

25   (Figures 3 and 4).

EXAMPLE 3

TH-1177 inhibited prostate cancer cell
proliferation *in vitro* by a cytostatic mechanism.

Inhibition of Ca^{2+} entry has been shown to limit
proliferation of cancer cells *in vitro*. The ability of
TH-1177 to block Ca^{2+} entry induced by release of Ca^{2+} from
internal stores such as that stimulated by engagement of
the P2 purinergic receptor suggested the possibility that
this agent could inhibit proliferation of prostate cancer
cells *in vitro*.

As shown in Figure 6, TH-1177 caused a
concentration dependent inhibition of the proliferation
of both LNCaP and PC3 cells. The IC_{50} for inhibition of
LNCaP proliferation was 4μM (Figure 6A) while the value
for PC3 prostate cancer cells was 14 μM (Figure 6B).
When compared to the IC_{50} values for inhibition of Ca^{2+}
entry of 3μM and 16μM for LNCaP and PC3 cells,
respectively, it was clear that TH-1177 inhibited
proliferation at a concentration that was similar to that
which was needed to block Ca^{2+} entry in these two cell
types.

Most conventional cancer chemotherapeutic drugs
are cytotoxic and exert their therapeutic benefit by
killing cancer cells. On the other hand, agents that act
by inhibition of Ca^{2+} entry (i.e. cytostatic agents) would
likely arrest cell proliferation rather than induce cell
death Meldolesi, J. (1995) Nat. Med., 1:512-513. To
address this possibility, LNCaP (Figure 7) prostate
cancer cells were allowed to grow unimpeded or exposed to
TH-1177 for 2 or 3 days before the agent was washed away.
Both cell lines grew in the absence of TH-1177 while
growth was stopped when the compound was present.

-54-

Removal of TH-1177 after exposure for 2 or 3 days was associated with resumption of a rate of growth that was similar to that seen with cells never exposed to TH-1177 (Figure 7). Similar results were seen with PC3 prostate cancer cells. Therefore, prostate cancer cells were quiescent in the presence of TH-1177 relative to cells that were cultured in the absence of this agent.

The experiment depicted in Figure 7 demonstrated that TH-1177 did not induce any long-lasting alteration in the proliferative phenotype of prostate cancer cells. The experiment depicted in Figure 8 was performed to further evaluate this possibility.

LNCaP cells were grown in the continuous presence of TH-1177 at one of three concentrations or in the compound's absence for 4 hr. The cell culture medium was then replaced with medium free of TH-1177 and maintained for an additional 48 hr. TH-1177 was then added to all cell cultures at the concentrations indicated in Figure 8 such that previously treated cell cultures received an identical second treatment. Prior treatment with TH-1177 had no effect on the response to the second exposure of this compound (Figure 7). This observation demonstrated that TH-1177 was present in order to inhibit proliferation and that TH-1177 did not alter the drug-sensitive phenotype of these human prostate cancer cell lines.

-55-

EXAMPLE 4

Inhibition of lymphocyte prostate and breast cancer cell proliferation in vitro.

As shown in Figures 10-29, TH-1087, TH-1113, TH-1211, and TH-1205 caused concentration dependent inhibition of the proliferation of Jurkat, PC3, LNCaP, MDA-468 and MDA-361 cells.

As shown in Figures 30-39, MMR-64, MMR-70 caused inhibition of the proliferation of Jurkat, PC3, L-NCaP, MDA-468 and MDA-361 cells.

EXAMPLE 5

TH-1177 slowed prostate cancer progression *in*

vivo. To begin an investigation of the possibility that
TH-1177 possesses *in vivo* activity against prostate
cancer, SCID mice were inoculated with PC3 cells by IP
injection. One day later, daily IP injections of TH-1177
or vehicle alone were begun. TH-1177 was administered at
doses or either 3 mg/kg or 10 mg/kg. These doses were
selected based upon the general range of doses for Ca^{2+}
channel blockers given by mouth to patients for the
treatment of hypertension. Daily dosing was also
selected based upon the general desirability of a once-a-
day treatment regimen. As shown in Figure 9, there was a
dose dependent increase in longevity associated with TH-
1177 administration. Life span was increased by 34%
(p=0.047) by TH-1177 at a dose of 3 mg/kg/d and by 38%
(p=0.0044) at a dose of 10 mg/kg/d.

Although the experiment of Figure 9 suggested a
lack of toxicity associated with TH-1177 administration,
the drug was further examined specifically for possible
toxicity. Four SCID mice without tumors were given TH-
1177 at a dose of 180 mg/kd/d. Treated mice were all
well groomed and active during treatment and no gross
abnormalities were noted at necropsy. Samples of kidney,
adrenal gland, heart and liver were within normal limits
on histological examination including active hepatic
hematopoiesis.

EXAMPLE 6

Using the measurement cell proliferation assay described hereinabove, the cell lines Jurkat, LNCap (prostate cancer cell), PC-31 (prostate cancer cell),

5 DU145 (prostate cancer cell), MDA-468 (breast cancer cell), MDA-36 (Breast cancer cell), MCF-7 (breast cancer cell), MIA PaCa-2 (pancreatic cancer cell), Hep G2 (liver cancer cell), A549 (non-small cell lung cancer cells), NCI H460 (non-small cell lung cancer cells), HT-29 (colon

10 cancer cell), HCT-116 (colon cancer cell) and SK OV-3 (ovarian cancer cell) were cultured in the presence of TH-1177, TH-1205, TH-1201, TH-2109, TH-2029, TH-2043, TN-2085 and TH-2279. IC_{50} values in µM are tabulated hereinbelow:

15

PROLIFERATION IC$_{50}$														
DRUG	Jurkat	LNCaP	PC-3	DU 145	MDA-468	MDA-361	MCF-7	MIA PaCa-2	Hep G2	A549	NCI H460	HT-29	HCT 116	SK-OV-3
TH-1177	8	4	15	19	13	26	21	17	32	71	15	~100	37	25
TH-1205	11	5	23	21	13	19	70	17	40	93	35	~100	30	33
TH-1211	4	3	17	13	11	14	24	17	20	41	13	~90	32	23
TH-2019	7	4	19	28	21	23	30	17	30	72	12	~100	31	30
TH-2029	11	5	18	17	10	16	37	17	30	26	24	~60	18	24
TH-2043	8	4	14	9	8*	13	29	17	>100	20	10	>100	10	23
TH-2085	10	4	36	41	14	26	33	17	28	90	31	~60	50	80
TH-2279	~50	~50	>100	>100	~100	>100	>100	>100	>100	>100	>100	>100	>100	>100

IC$_{50}$ values in µM.

-59-

EXAMPLE 7

Repeating the cell proliferation test of Example 6, TH-3101, TH-3104 and TH-3105 were each tested on various cell lines: Jurkat, LNCap and MDA-361. The results are tabulated hereinbelow:

	Jurkat	LNCaP	MDA-361
TH-3101	4.6 µM	4.1 µM	7.3 µM
TH-3104	3.8 µM	2.4 µM	13.0 µM
TH-3105	5.0 µM	3.2 µM	29.0 µM

EXAMPLE 8

The IC_{50} values for TH-1177 and other related drugs in the proliferation test on LNCaP were plotted against IC_{50} values for Inhibition of Calcium Influx (obtained in accordance with the procedure described hereinabove of measurement of intracellular calcium concentration) with respect to LNCaP cells.

The results are graphically depicted in Figure 40. The graph clearly shows that two functions are correlated in a linear fashion.

EXAMPLE 9

Xenopus oocytes were transfected with the α1H
subunit of a calcium channel of a human heart prepared in
accordance with the procedure described by Cribbs, et al.

5   in <u>Circulation Research</u>, <u>1998</u>, 83, 103-109, the contents
of which are incorporated by reference. The α1H plasmid
was obtained by cloning the α1H sequence into the EcoRV-X
baI site of the transfection vector pcDNA3, in accordance
with the procedure described in the Cribbs, et al.

10   article. Xenopus oocyte cells (1 x 10^5 per 35 mm dish)
were transfected with 2 µg of alpha 1H-Tx plasmid. The
transfected cells were depolarized with standard
depolarizing bath solution containing (mmol/L) KCl 140,
EGTA 10, $MgCl_2$ 1, $CaCl_2$ 1, dextrose 10, and HEPES 10 (pH

15   6.4). Whole cell recording was performed, with Ba^{2+} used
as the charge carrier, by the ruptured patch method in a
solution containing (mmol/L) $BaCl_2$ 10, TEA-Cl 140, CsCl
6, and HEPES 10 (pH 7.4 adjusted with TEA-OH). The
internal pipette solution contained (mmol/L) CsCl 55, CS

20   SO_3 75, $MgCl_2$ 10, EGTA 0.1, and HEPES 10 (pH adjusted to
7.2 with (CsOH). Currents were recorded using an
Axopatch 200A, a Digidata 1200 A/D converter and pCLAMP
software (Axon Instruments, Inc.).

The current of the cell in the depolarized cell

25   was recorded over time (control). Within a few minutes
after the control plot was recorded, the cells were again
depolarized, but this time, the bath contained in
addition 10 µM TH-1177. The current was recorded for the
same length of time as the control. The current was

30   recorded at about zero pA. Then the cells in the bath
containing 10µM TH-1177 were depolarized again, but this
time the bath medium containing TH-1177 was aspirated out

and replaced with fresh calcium medium depolarizing medium and the current was measured.

The results are graphically depicted in Figure 41.

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The experiment clearly illustrates that TH-1177 inhibits the ability of calcium to cross the cell membrane in these cells. Allowing TH-1177 to wash out restores the ability of calcium to cross the cell membrane.

EXAMPLE 10

The procedure of Example 9 was repeated except the cells were transfected with 2 µg of α1G subunit prepared in accordance with the procedure described in Cribbs, et al., _FEBS Letters_, 66, 54-58 (2000). Here the cells were depolarized using the depolarizing medium of Example 9. When the current reached the maximum level, then 10 µm pH 1177 was added to the medium and the change in current was observed. The current was measured at about zero pA. When the current remained constant, the TH-1177 was washed out, in accordance with the procedure in Example 9 and the change in the current value was again recorded.

The change in the current was plotted and the plot is depicted in Figure 42.

This experiment also shows that TH-1177 completely inhibits the ability of calcium to cross the cell membrane in the Xenopus oocyte cell transfected with the α1G subunit. Allowing TH-1177 to wash out restore the ability of calcium to cross the cell membrane.

The data in Examples 9 and 10 clearly demonstrate that compounds of the present invention, e.g., TH-1177 and its cogeners inhibit T-like calcium channels.

The above preferred embodiments and examples were given to illustrate the scope and spirit of the present invention. These embodiments and examples will make apparent to those skilled in the art other embodiments and examples. The other embodiments and examples are within the contemplation of the present invention. Therefore, the present invention should be limited only by the amended claims.

5

1. A compound of the formula:
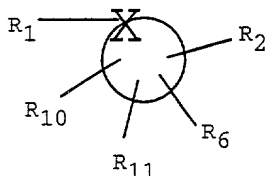
and pharmaceutically acceptable salts thereof
wherein
X is N or CH;

 is a cyclic 5-10 membered cyclic ring which

is saturated and which may contain 1 or 2 additional ring
heteroatoms selected from the group consisting of O, S
and N, with the remaining ring atoms being carbon atoms;

R_1 is $(CH_2)n - Z - (R_5)$, Q, hydrogen or lower
alkyl;

R_2 is hydrogen or Q;

Q and Q' may be the same or different and are
independently
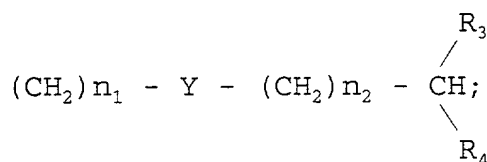
$$(CH_2)n_1 - Y - (CH_2)n_2 - \overset{\displaystyle R_3}{\underset{\displaystyle R_4}{CH}};$$

Z is a chemical bond, CH_2, O, S or NH;

Y is CH_2, O, S or NH;

R_3, R_4 and R_5 are independently cyclic rings
containing 6-14 ring carbon atoms, and containing no
hetero ring atoms, which cyclic rings may be completely
saturated, partially unsaturated or aromatic, and which

-66-

are unsubstituted or substituted with an electron donating group or electron withdrawing group;

R_3 and R_4 may be fused to form a cyclic ring structure containing 12-28 carbon atoms;

R_6, R_{10} and R_{11} are independently hydrogen or lower alkyl, which is unsubstituted or substituted with an electron withdrawing group or electron donating group;

n_2 is 0-8; and

n and n_1 are independently 1-8, provided that either R_1 is Q or R_2 is Q'.

2. The compound according to Claim 1 wherein Y is O or CH_2.

3. The compound according to Claim 1 wherein n_2 is 0.

4. The compound according to Claim 1 wherein n_1 is 1 and n_2 is 0.

5. The compound according to Claim 1 wherein R_1 is $(CH_2)_n - Z - R_5$, hydrogen or lower alkyl and R_2 is Q'.

6. The compound according to Claim 1 wherein R_1 is Q and R_2 is Q'.

7. The compound according to Claim 5 having the formula:
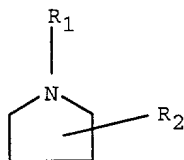
8. The compound according to Claim 7 having the formula:

-67-

$$R_1 \text{—N} \overbrace{\hspace{1cm}}^{} \text{—}R_2$$
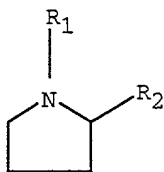
9. The compound according to Claim 7 or 8 wherein Y is 0.

10. The compound according to any one of Claims 7, 8 or 9 wherein R_3 and R_4 are independently aromatic rings.

11. The compound according to Claim 5 having the formula:
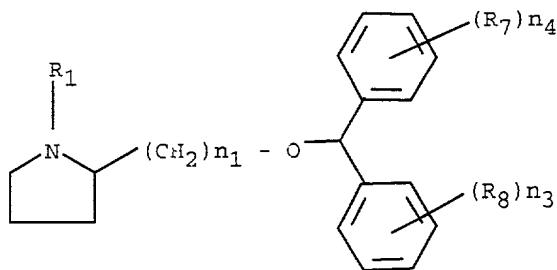
wherein

n_3 and n_4 are independently 1-5;

n_1 is 1-8;

R_1 is $(CH_2)n - Z - R_5$, hydrogen or lower alkyl, R_7 and each R_8 are independently hydrogen, an electron donating or electron withdrawing group;

Z is CH_2, O, S or NH; and

R_5 is a cyclic ring containing 6-14 ring carbon atoms and is aromatic and may be unsubstituted or substituted with an electron withdrawing group or electron donating group.

-68-

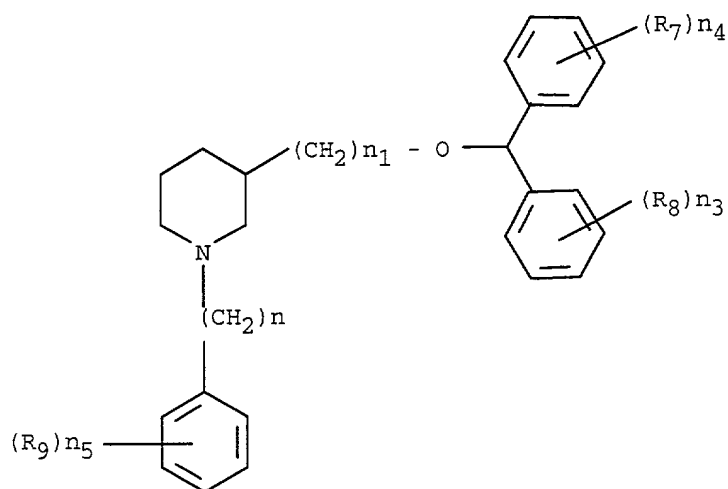12. The compound according to Claim 11 having the formula:
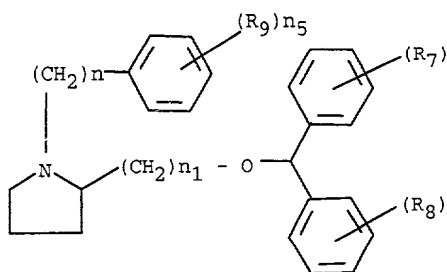
wherein

R$_9$ is hydrogen, an electron donating group or electron withdrawing group and;

n$_5$ is 1-5.

13. The compound according to Claim 1, 10 or 12 wherein n is 1.

14. The compound according to any one of Claims 1, 11 or 12 wherein the electron donating group is amino, hydroxy or lower alkoxy, and the electron withdrawing group is halo, lower alkyl, nitro or nitrile.

15. The compound according to Claim 14 wherein the electron donating group is lower alkoxy and the electron withdrawing group is halo.

16. The compound according to Claim 11 or 12 wherein R$_7$ and R$_8$ are halo.

17. The compound according to Claim 1 having the formula:

-69-
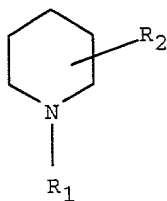
18. The compound according to Claim 17 having the formula:

or
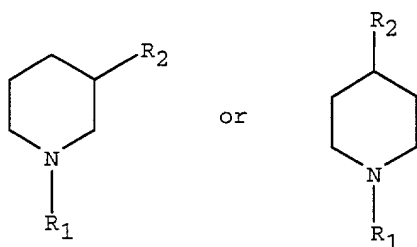
19. The compound according to Claim 17 or 18 wherein Y is O.

20. The compound according to Claim 17, 18 or 19 wherein R_3 and R_4 are independently aromatic.

21. The compound according to Claim 17 having the formula:
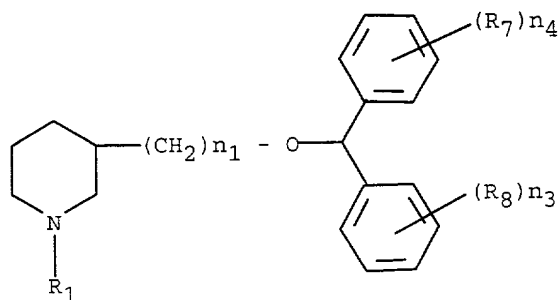
wherein

n_3 and n_4 are independently 1-5;

n_1 is 1-8;

R_1 is $(CH_2)n - Z - R_5$, hydrogen or lower alkyl;

each R_7 and each R_8 are the same or different

-70-

and are independently hydrogen, an electron donating or electron withdrawing group;

Z is CH_2, O, S or NH;

R_5 is a cyclic ring containing 6-14 ring carbon atoms and is aromatic and may be unsubstituted or substituted with an electron withdrawing group or electron donating group.

22. The compound according to Claim 17 having the formula:
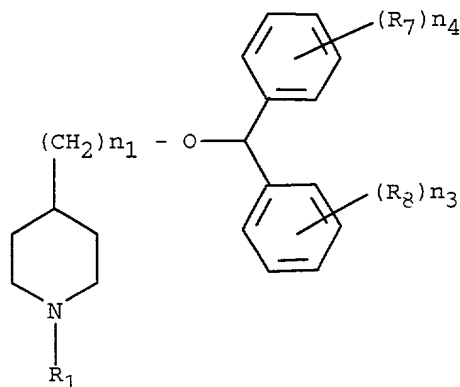
wherein

n_3 and n_4 are independently 1-5;

n_1 is 1-8;

R_1 is $(CH_2)n$ - Z - R_5, hydrogen or lower alkyl;

each R_7 and each R_8 are the same or different and are independently hydrogen, an electron donating or electron withdrawing group;

Z is CH_2, O, S or NH;

R_5 is a cyclic ring containing 6-14 ring carbon atoms and is aromatic and may be unsubstituted or substituted with an electron withdrawing group or electron donating group.

-71-

23. The compound according to Claim 22 having the formula:
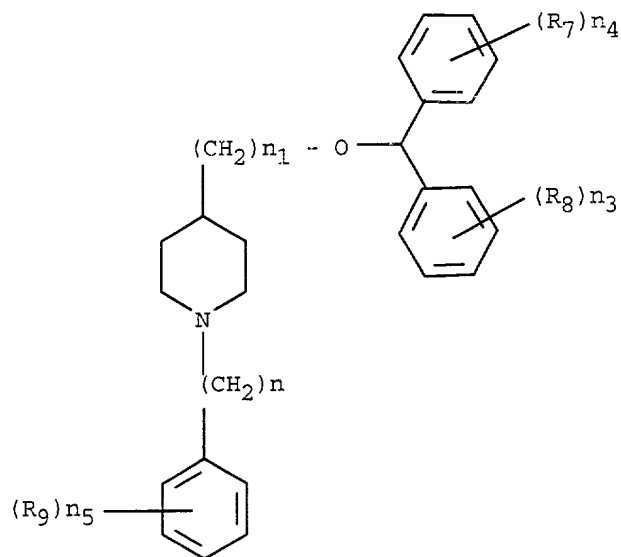
wherein

each R_7 and each R_8 are the same or different and are independently hydrogen, an electron donating or electron withdrawing group;

R_9 is hydrogen, an electron donating group or electron withdrawing group;

n_3, n_4 and n_5 are independently 1-5; and

n and n_1 are independently 1-8.

24. The compound according to Claim 21 having the formula:

wherein

each R_7 and each R_8 are the same or different and are independently hydrogen, an electron donating or electron withdrawing group;

R_9 is hydrogen, an electron donating group or electron withdrawing group;

n_3, n_4 and n_5 are independently 1-5; and

n and n_1 are independently 1-8.

25. The compound according to any one of Claims 21-24 wherein n_1 is 1.

26. The compound according to any one of Claims 21-24 wherein the electron donating group is amino, hydroxy or alkoxy and the electron withdrawing group is halo, lower alkyl, nitro or nitrile.

27. The compound according to Claim 18 having the structure

5
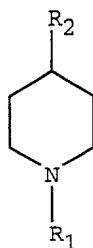
10   wherein

R_1 is Q and R_2 is hydrogen or Q'.

28. The compound according to Claim 27 wherein R_1 is

15

$$(CH_2)n - (CH) \begin{array}{c} R_3 \\ \diagup \\ \diagdown \\ R_4 \end{array}.$$
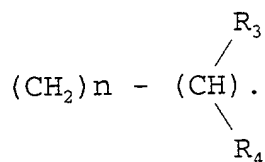
20   29. The compound according to Claim 28 wherein n is 1.

30. The compound according to any one of Claims 27-29 wherein R_3 and R_4 are independently phenyl.

31. The compound according to Claim 27 having

25   the structure

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-74-
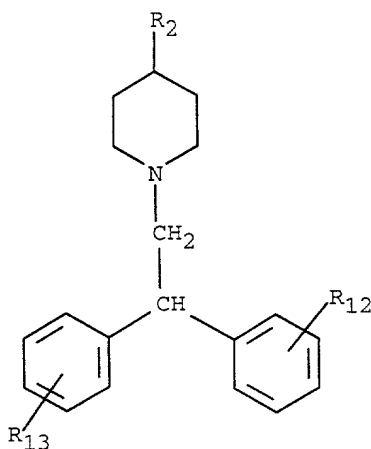
wherein

R$_{13}$ and R$_{12}$ are independently hydrogen, an electron donating group or an electron withdrawing group.

32. The compound according to Claim 31 wherein R$_2$ is H.

33. The compound according to Claim 31 wherein R$_2$ is

$$(CH_2)n_1 - Y - (CH_2)n_2 - CH \overset{\diagup R_3}{\underset{\diagdown R_4}{}}$$
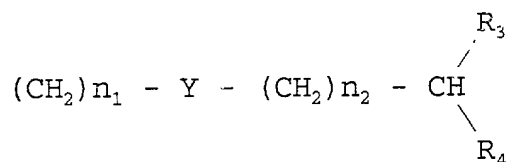
wherein

Y is O, S, NH or CH$_2$;

R$_3$ and R$_4$ are independently the same or different and are cyclic rings containing 6-14 ring carbon atoms, which cyclic rings are aromatic and which are unsubstituted or substituted with an electron donating group or an electron withdrawing group;

n$_1$ is 1-8, and n$_2$ is 0-8.

34. The compound according to Claim 33 wherein Y is O.

35. The compound according to Claim 33 or 34 wherein R$_3$ and R$_4$ are independently phenyl rings which are unsubstituted or substituted with an electron donating group or electron donating groups.

36. The compound according to Claim 33 wherein n$_1$ is 1.

37. The compound according to Claim 33 wherein n$_2$ is 0.

38. The compound according to Claim 33 wherein n$_1$ is 1 and n$_2$ is 0.

39. The compound according to Claim 31 having the formula:

-75-

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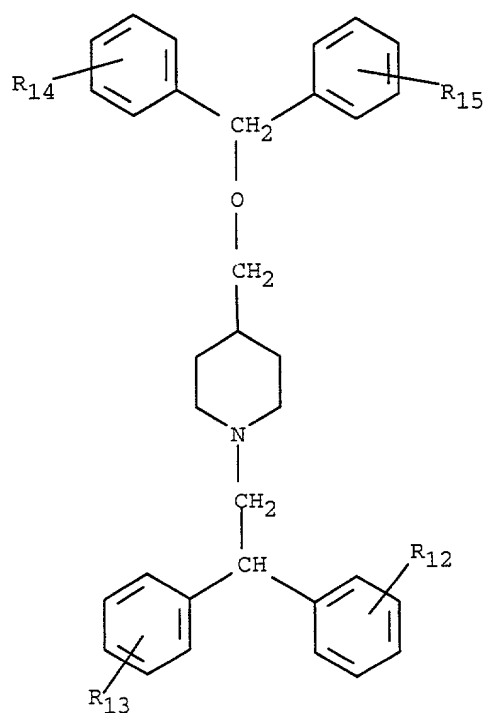
wherein

R_{12}, R_{13}, R_{14} and R_{15} are independently hydrogen, electron withdrawing group or electron donating group.

40. A stereoisomer of the compound of Claim 1.

41. A pharmaceutical composition comprising a cytostatic effective amount of a compound according to Claim 1 and a pharmaceutically acceptable carrier therefor.

42. A method of inhibiting cancer cell proliferation in a mammal in need of such treatment comprising administering to said mammal a cytostatic effective amount of a compound according to any one of Claims 1, 7, 8, 11, 12, 17, 18, 21, 22, 23, 24 or 31.

43. The method according to Claim 42 wherein said mammal is a human.

44. The method according to Claim 43 wherein the compound is administered in amounts ranging from about .5 mg to about 100 mg/kg of body weight per day.

45. A method of treating cancer in a mammal in need thereof comprising administering to said mammal an effective amount of a compound according to any one of Claims 1, 7, 8, 11, 12, 17, 18, 21, 22, 23, 24 or 31.

46. A substantially pure compound of Claim 1.

47. A method of treating cancer in an animal afflicted with such disease which comprises administering to an animal in need of such treatment an organic calcium blocker that inhibits the entry of calcium ions across the cell membrane through a T-like calcium channel in cancer cells in response to a mitogenic stimulus, said calcium blocker being present in an amount effective to inhibit the passage of calcium into the cell.

48. The method according to Claim 47 in which the compound blocks calcium entry into the cell by interacting with the $\alpha 1$ subunit of a calcium channel.

49. The compound according to Claim 1 which contains 1 additional heteroatom selected from the group consisting of O and N.

50. The compound according to Claim 1 which contains no additional heteroatoms.

51. The method according to Claim 48 wherein the $\alpha 1$ subunit is an $\alpha 1G$ or $\alpha 1H$

ABSTRACT

The present invention is directed to compounds useful as cancer cell inhibitors, compositions containing such compounds and methods for inhibiting proliferation of electrically non-excitable cells.
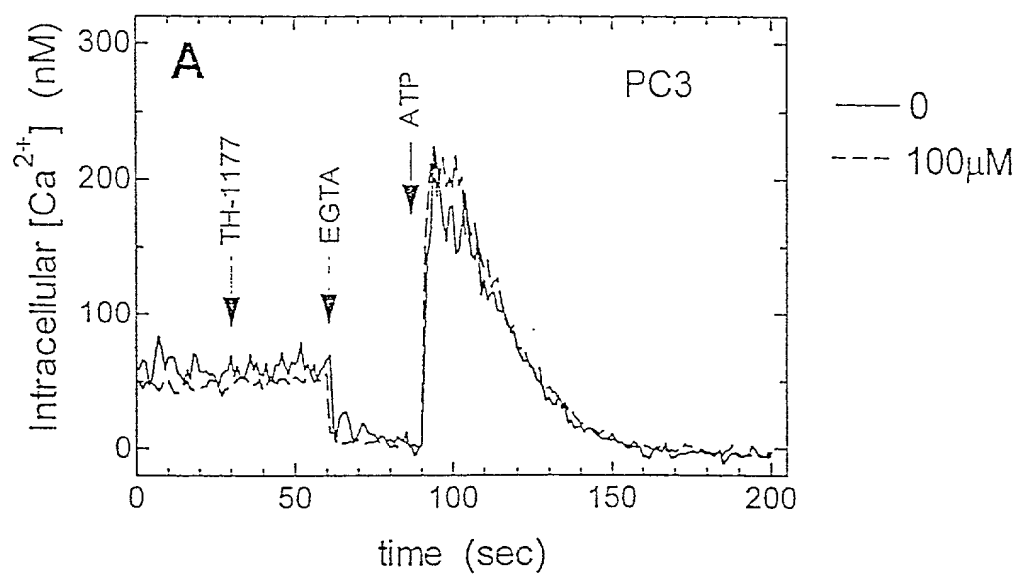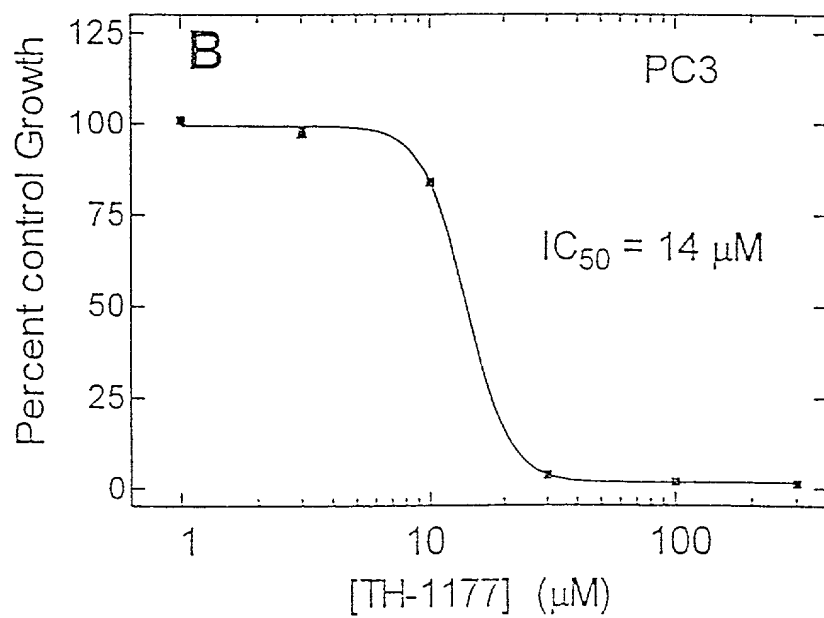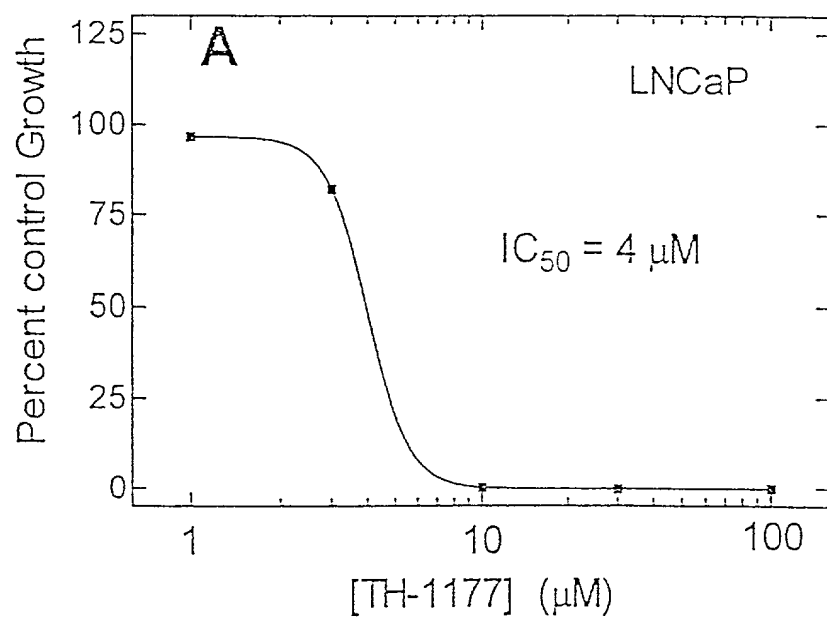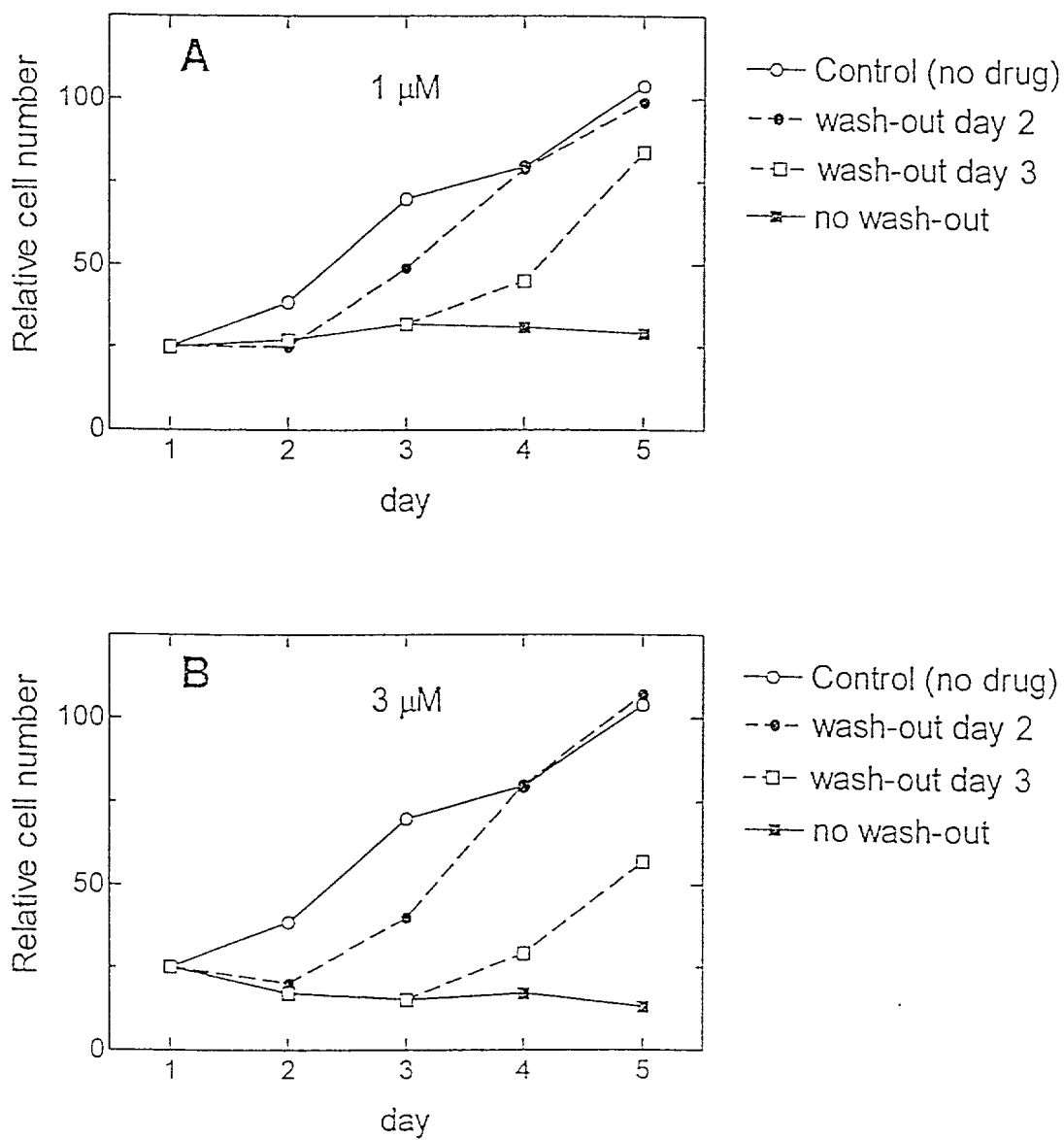
-78-
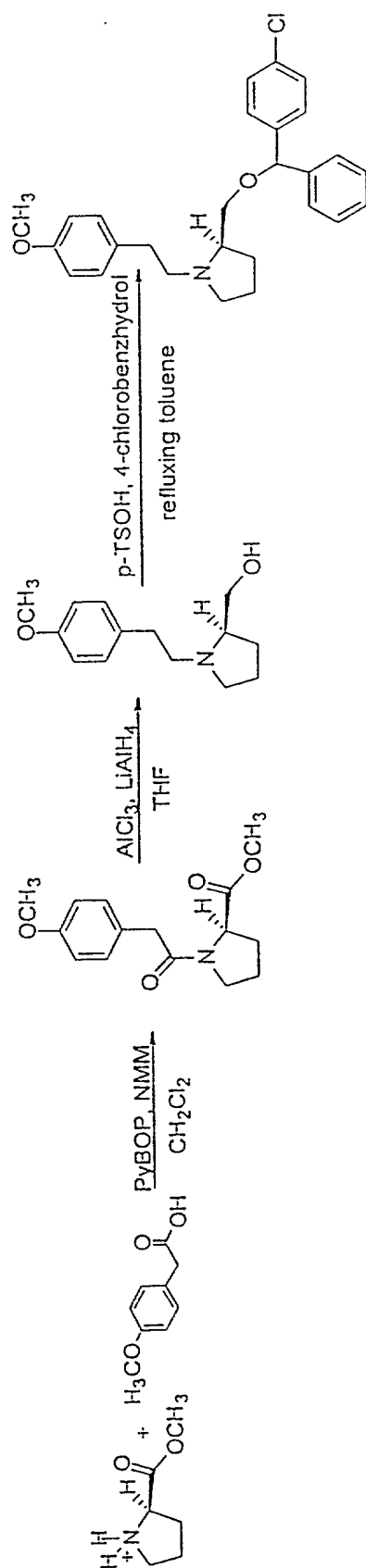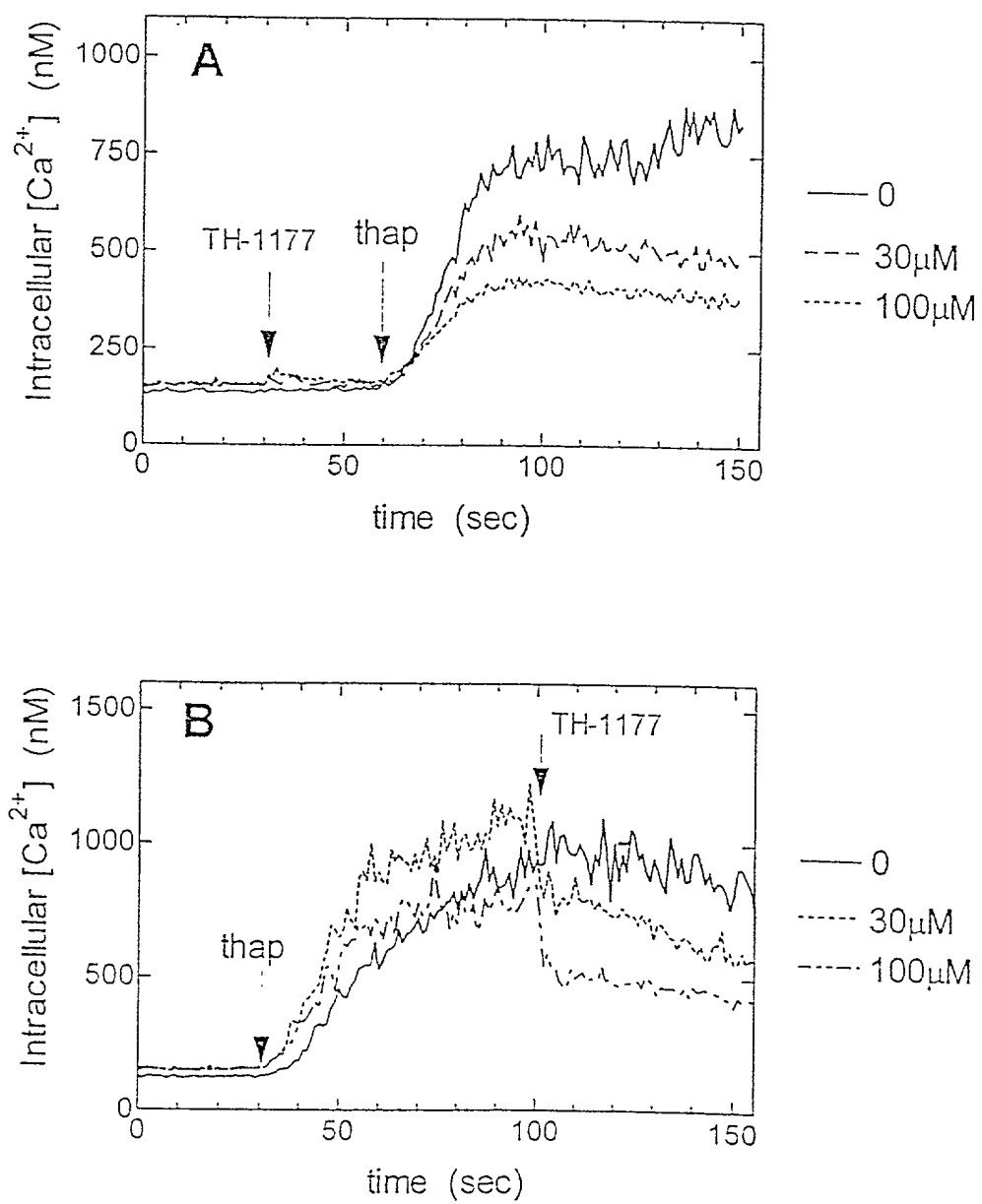
FIGURE 1
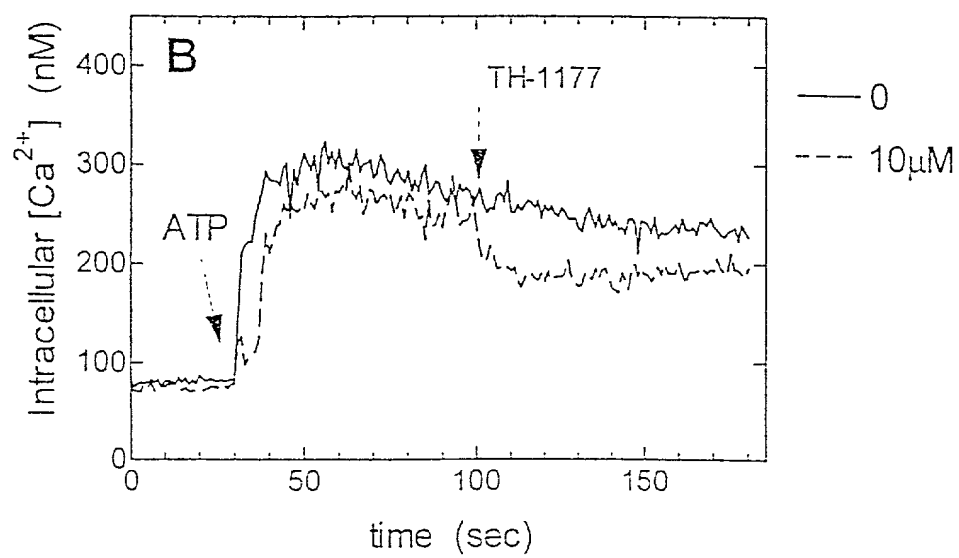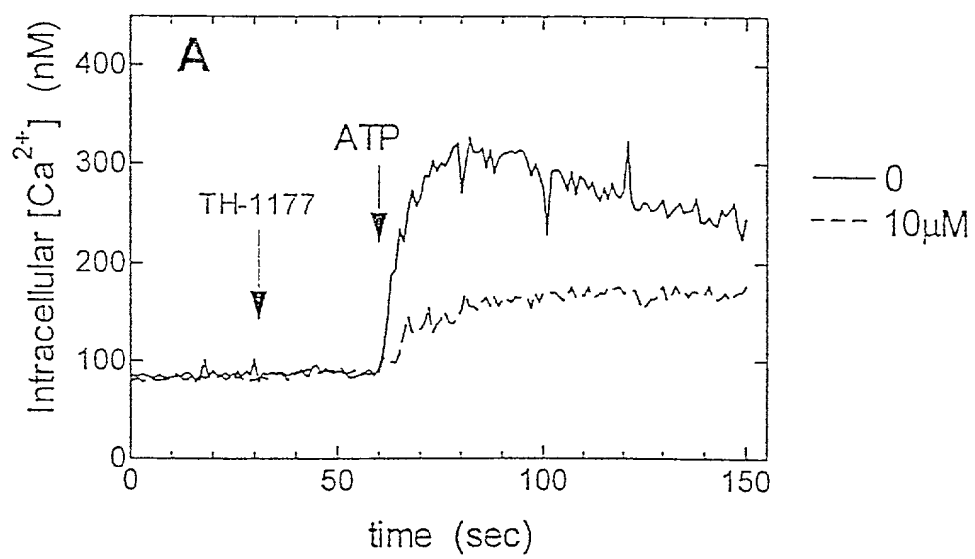
FIGURE 2

FIGURE 3

FIGURE 4
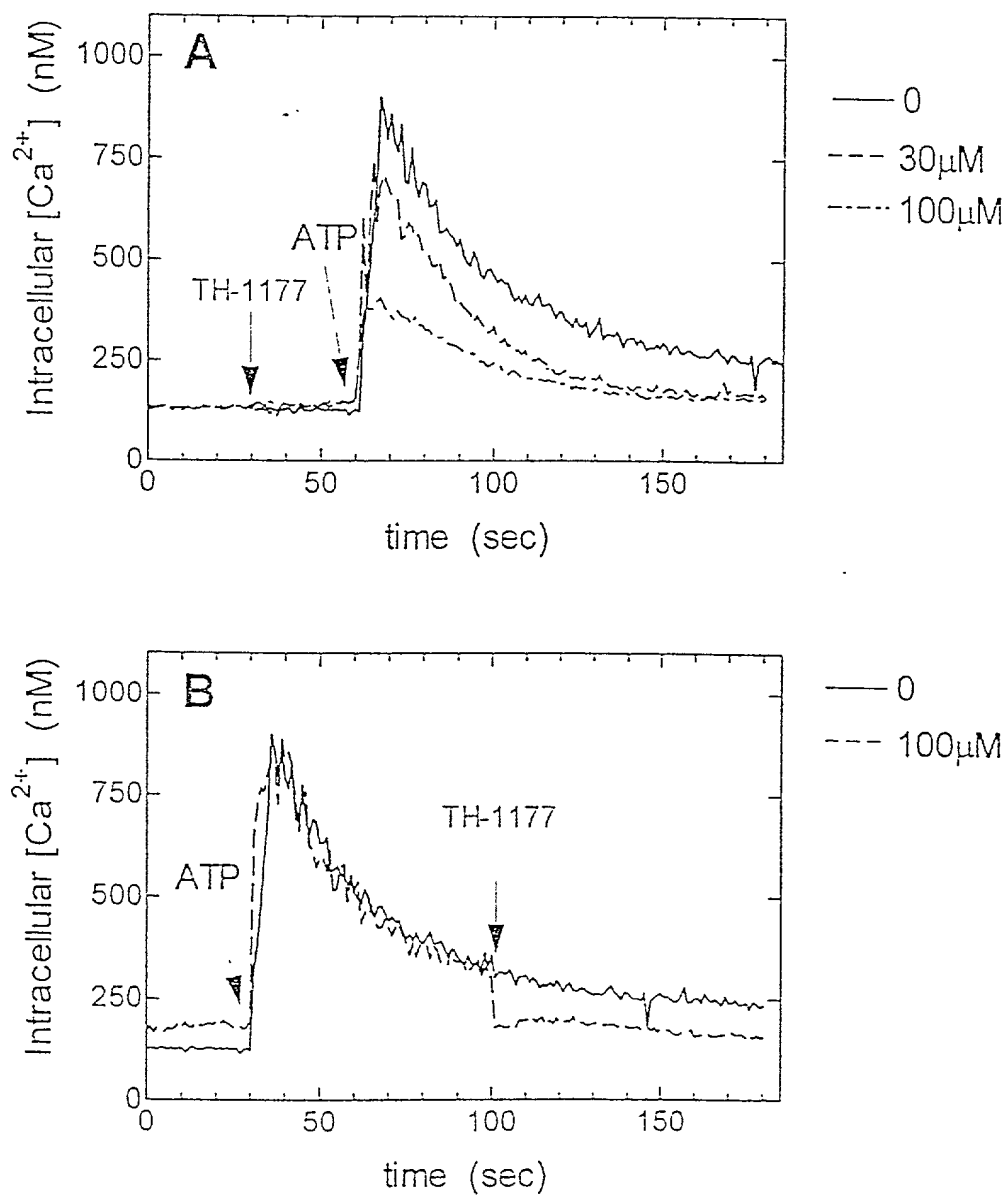
FIGURE 5
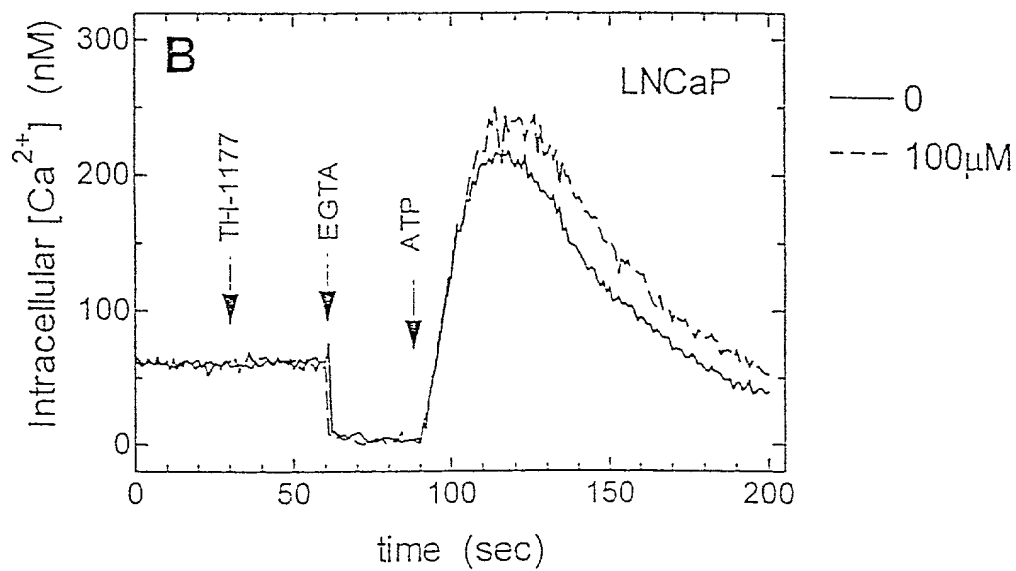
FIGURE 6

FIGURE 7

FIGURE 8
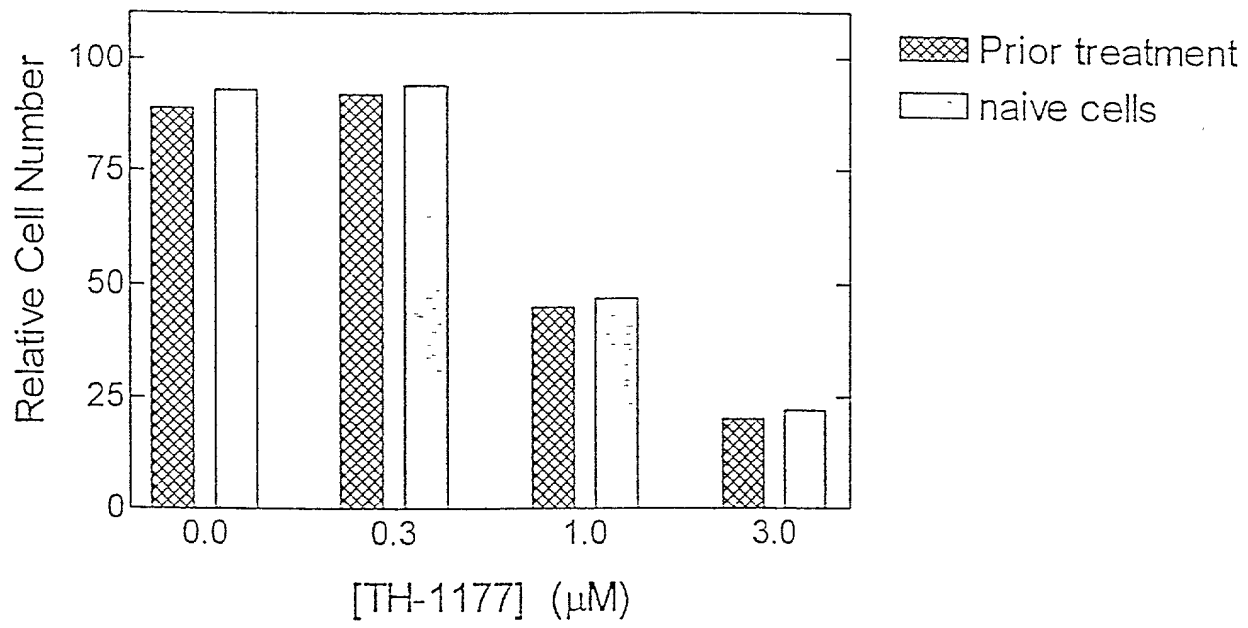
FIGURE 9
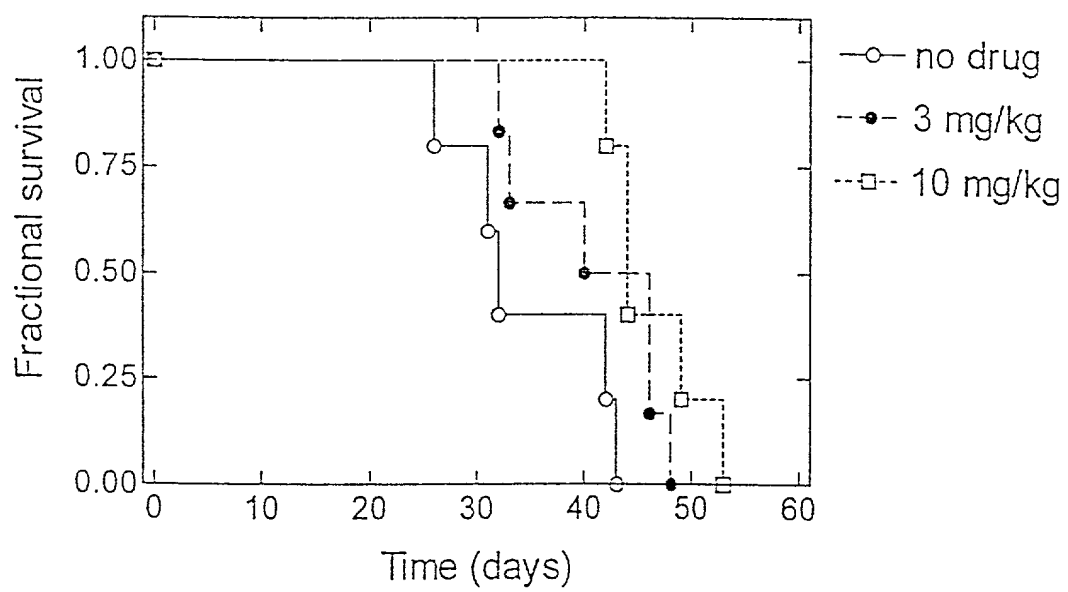
FIGURE 10

Jurkat - TH-1087

$IC_{50} = 8.3 \, \mu M$
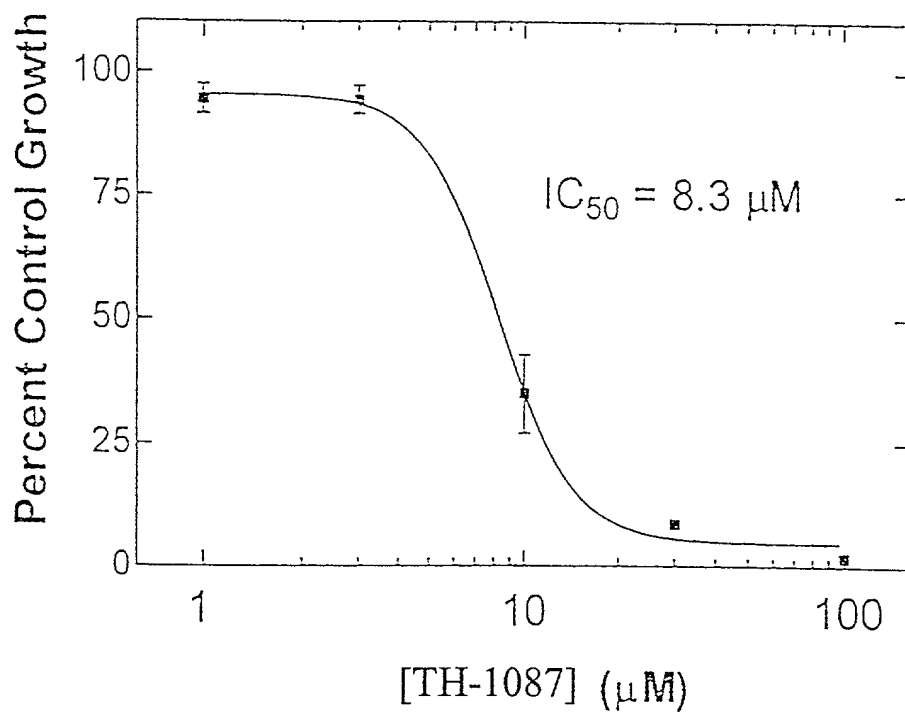
[TH-1087]	Jurkat		
	Y	SEM	N
100	2.0	0.14	6
30	8.5	0.96	6
10	34.9	7.85	6
3	94.4	2.96	6
1	94.5	3.14	6

FIGURE 11

PC3 - TH-1087

Graph: Percent Control Growth (y-axis, 0 to 100) vs [TH-1087] (μM) (x-axis, 1 to 100, log scale). $EC_{50} = 9.9\ \mu M$
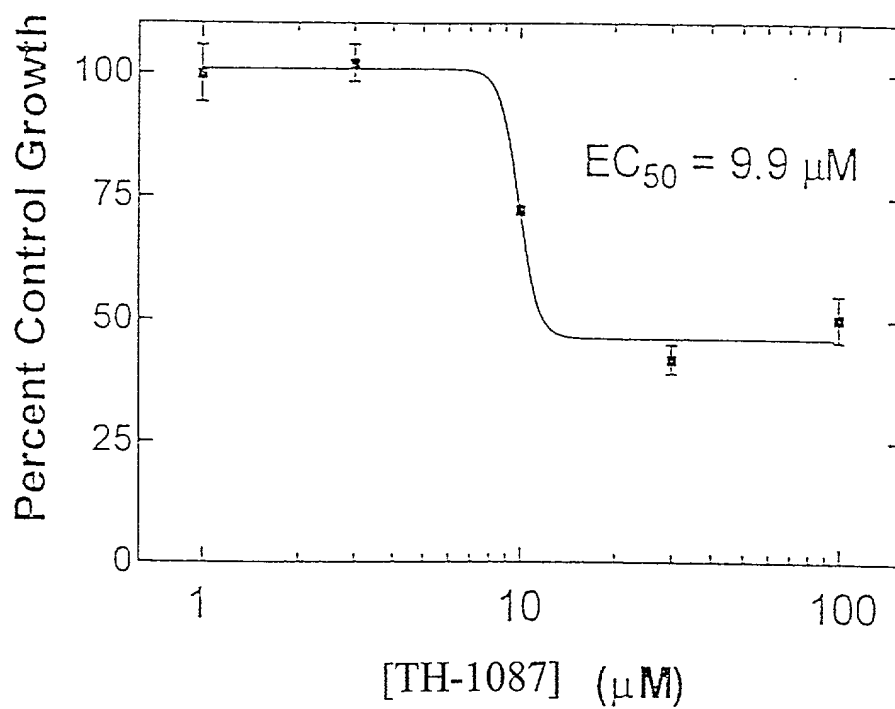
[TH-1087]	PC3		
	Y	SEM	N
100	50.4	4.84	6
30	41.8	3.02	6
10	72.3	0.95	6
3	102.0	3.73	6
1	99.8	5.77	6

FIGURE 12

LNCaP/TH-1087

$IC_{50} = 5\ \mu M$
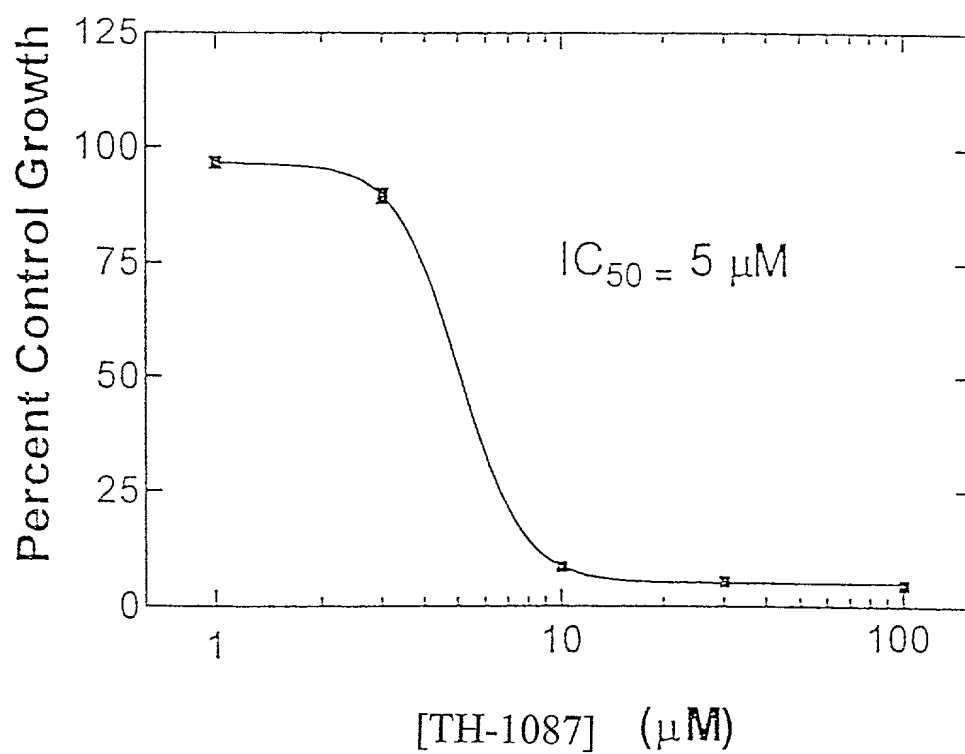
[TH-1087] (μM)

[TH-1087]	LNCaP		
	Y	SEM	N
1	96.8	1.29	3
3	89.7	1.60	3
10	8.5	0.06	3
30	5.4	0.19	3
100	4.7	0.15	3

FIGURE 13

MDA-468-TH-1087

Graph: Percent Control Growth vs [TH-1087] (μM). $EC_{50} = 10 \ \mu M$
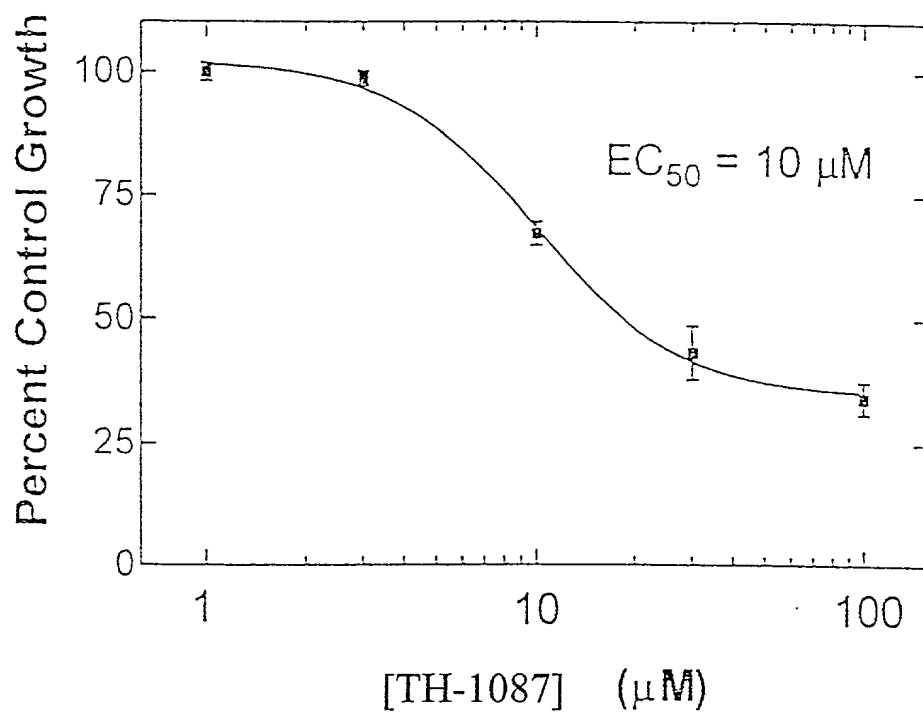
[TH-1087]	MDA-468		
	Y	SEM	N
100	34.2	3.26	6
30	43.3	5.42	6
10	67.3	2.44	6
3	98.8	1.42	6
1	100.0	1.77	6

FIGURE 14

MDA-361-TH-1087

EC$_{50}$ = 10.5 μM
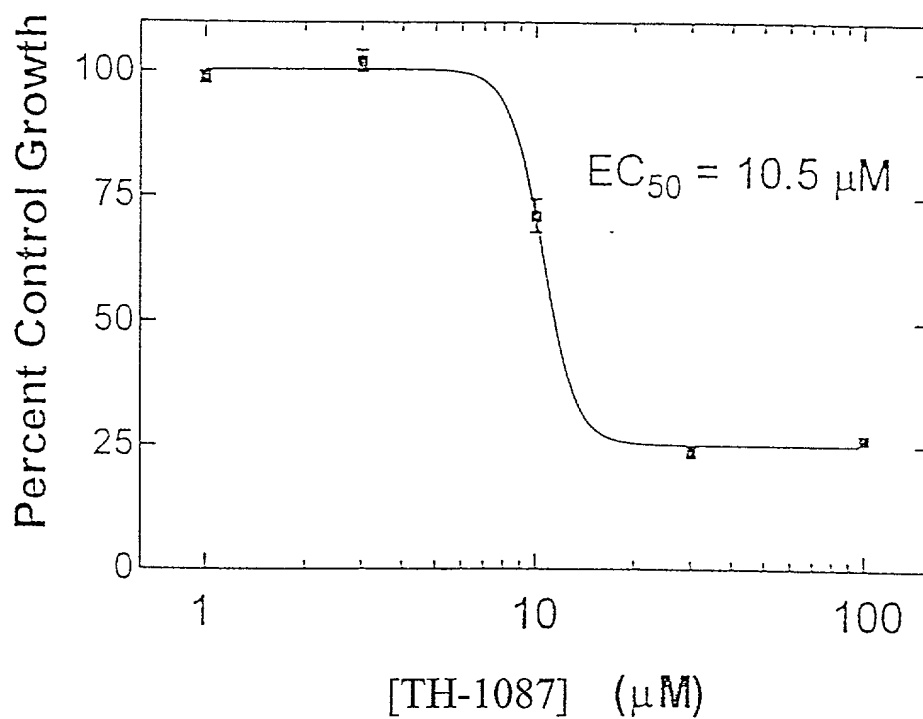
[TH-1087] (μM)

[TH-1087]	MDA-361		
	Y	SEM	N
100	26.5	0.57	6
30	23.7	0.52	6
10	71.0	3.33	6
3	102.0	2.19	6
1	98.8	1.23	6

FIGURE 15

Jurkat - TH-1113

Figure showing a dose-response curve with Percent Control Growth on the y-axis and [TH-1113] (μM) on the x-axis. $IC_{50} = 12.6\ \mu M$
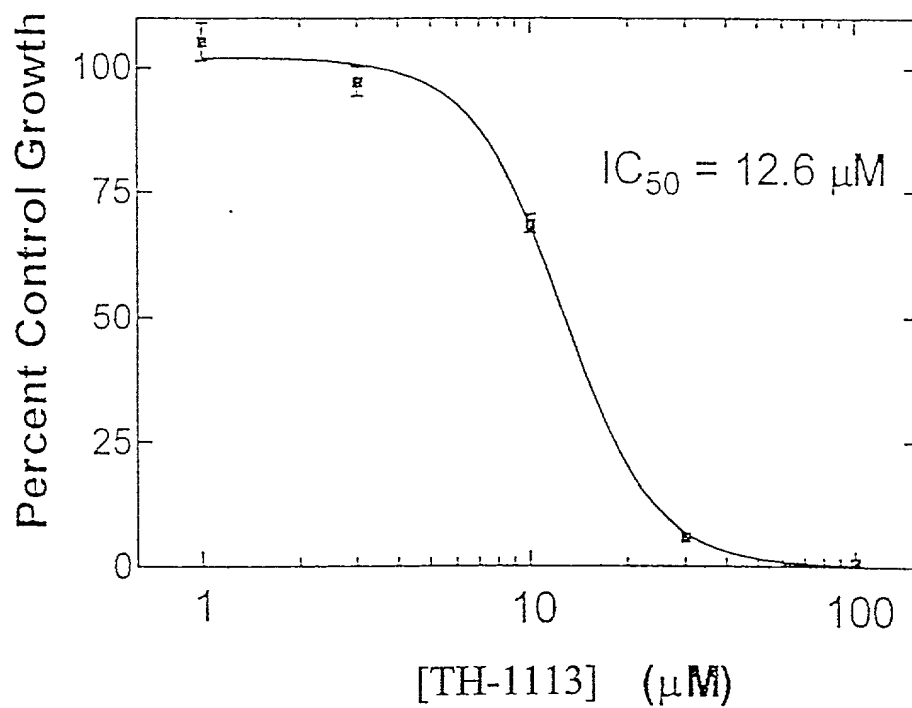
[TH-1113]	Jurkat		
	Y	SEM	N
0	95.4	2.49	9
0	104.6	2.71	9
100	1.0	0.73	9
30	6.1	0.58	9
10	68.7	1.81	9
3	97.2	3.01	9
1	105.2	3.82	9

FIGURE 16

PC3 - TH-1113

Percent Control Growth vs [TH-1113] (μM)

$IC_{50} = 89 \ \mu M$
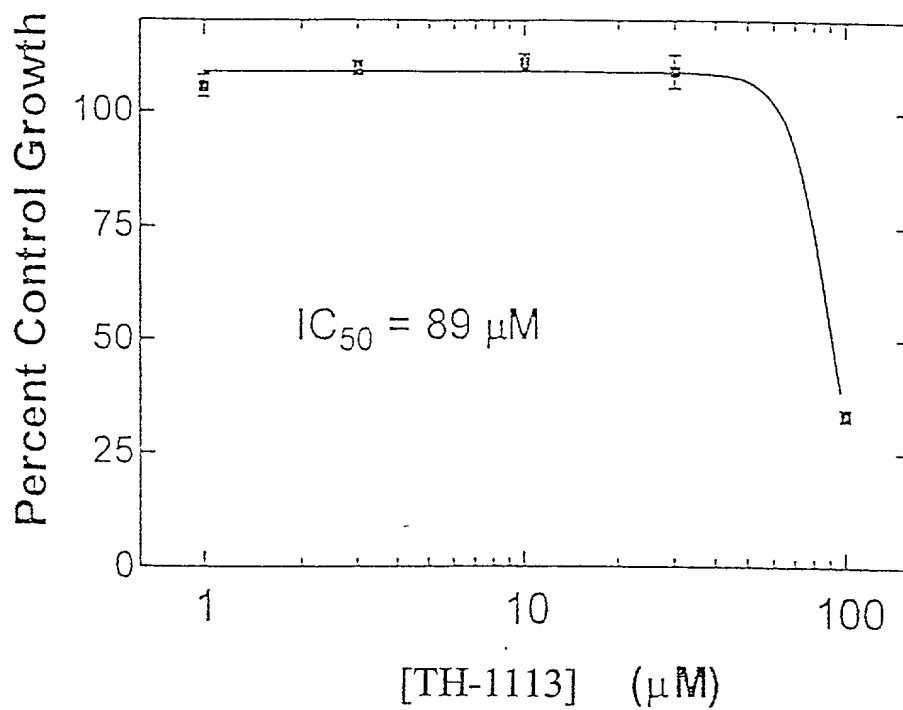
[TH-1113]	PC3		
	Y	SEM	N
0	106.7	3.34	6
0	93.3	2.44	6
100	33.4	1.41	6
30	109.0	3.56	6
10	110.8	1.83	6
3	109.5	1.56	6
1	105.6	2.34	6

FIGURE 17

LNCaP/TH-1113

$IC_{50} = 6 \ \mu M$
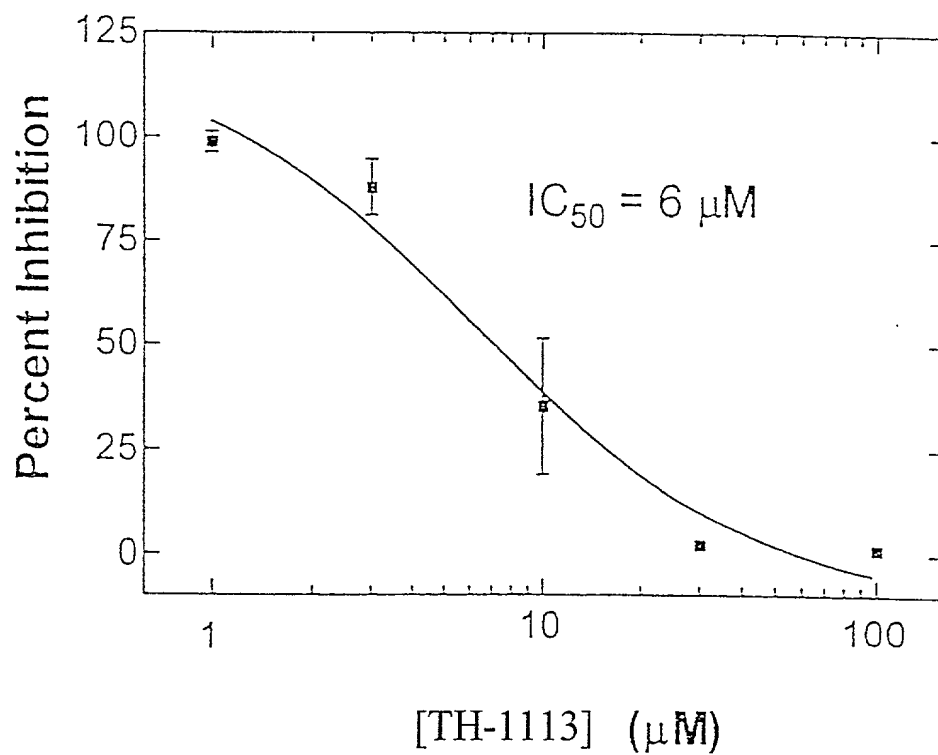
[TH-1113] (μM)

[TH-1113]	Data Set-A		
	Y	SEM	N
100	1.3	0.96	9
30	2.2	1.14	9
10	35.3	16.22	9
3	87.8	6.77	9
1	98.8	2.52	9

FIGURE 18
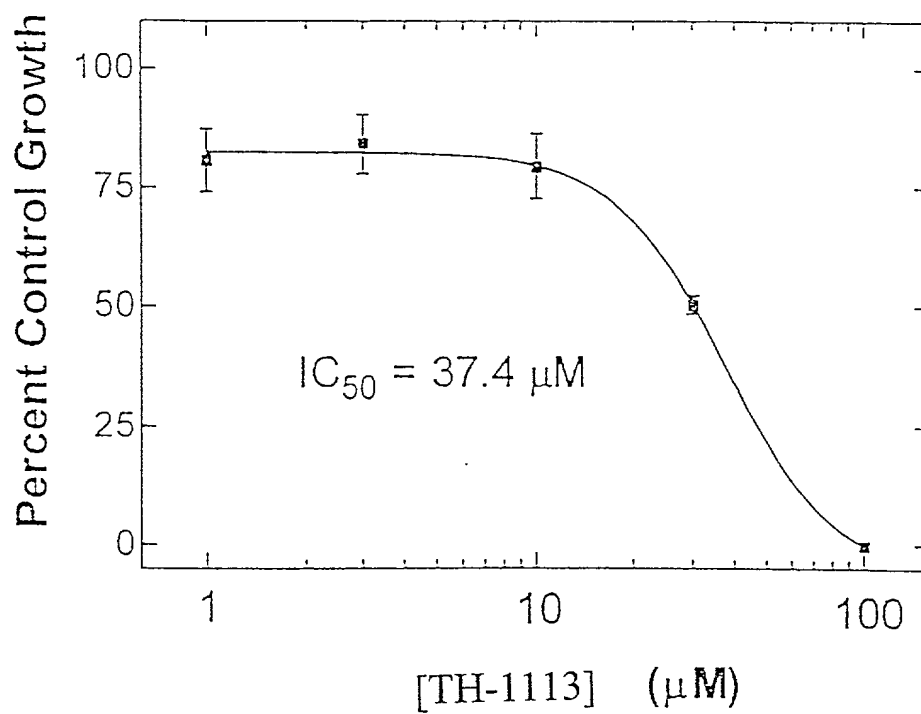
MDA-468-TH-1113

[TH-1113]	MDA-468		
	Y	SEM	N
0	101.5	2.40	6
0	98.5	1.69	6
100	-0.1	0.14	6
30	50.3	1.91	6
10	79.4	6.85	6
3	84.1	6.19	6
1	80.6	6.60	6

FIGURE 19

MDA-361-TH-1113

Figure shows a graph with Percent Control Growth (y-axis) versus [TH-1113] (μM) (x-axis), with IC$_{50}$ = 29.4 μM.
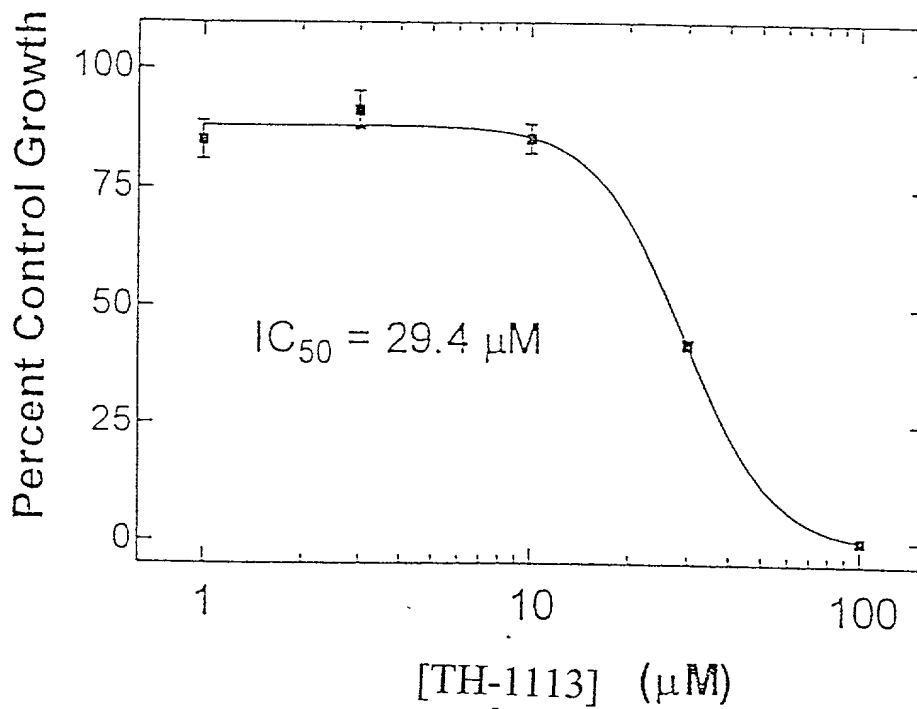
[TH-1113]	MDA-361		
	Y	SEM	N
0	108.4	2.09	6
0	91.7	0.46	6
100	0.1	0.29	6
30	41.8	1.11	6
10	85.5	3.11	6
3	91.3	4.03	6
1	85.0	4.08	6

FIGURE 20

Jurkat/TH-1211

$IC_{50} = 8.6 \ \mu M$

Percent Inhibition
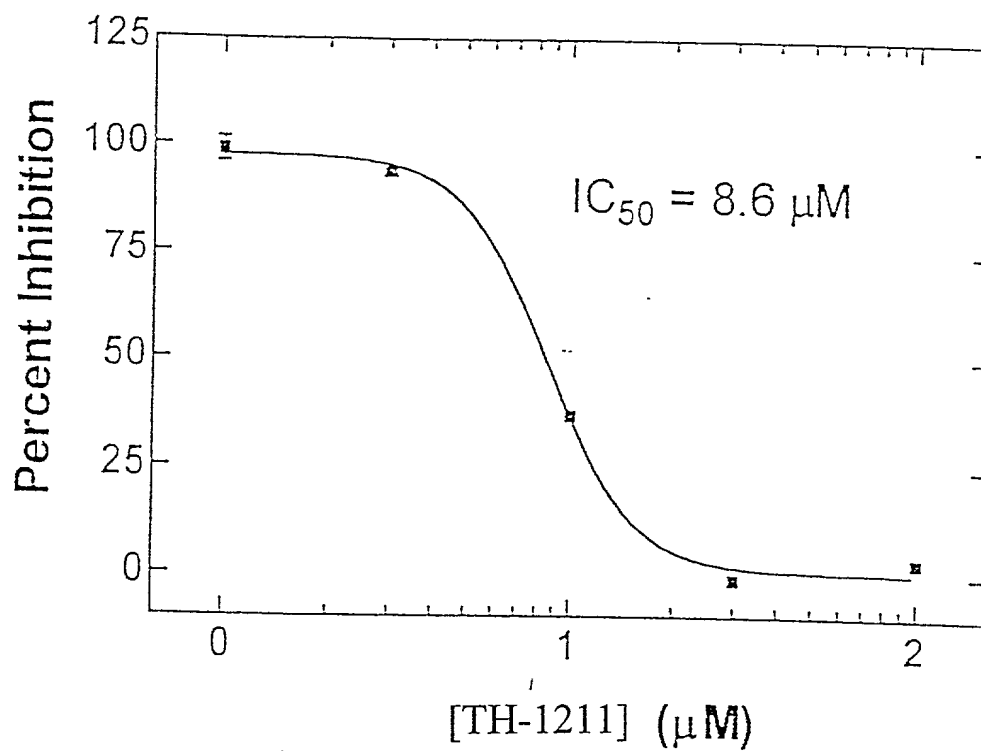
[TH-1211] (μM)

[TH-1211]	Jurkat	
	Y	SEM
100	3.1	0.49
30	-1.2	0.49
10	36.9	0.49
3	93.8	1.30
1	98.7	2.84

FIGURE 21

PC3/TH-1211

Graph: Percent Inhibition (y-axis, 0 to 125) vs [TH-1211] (µM) (x-axis, 1 to 100, log scale). IC$_{50}$ = 68 µM
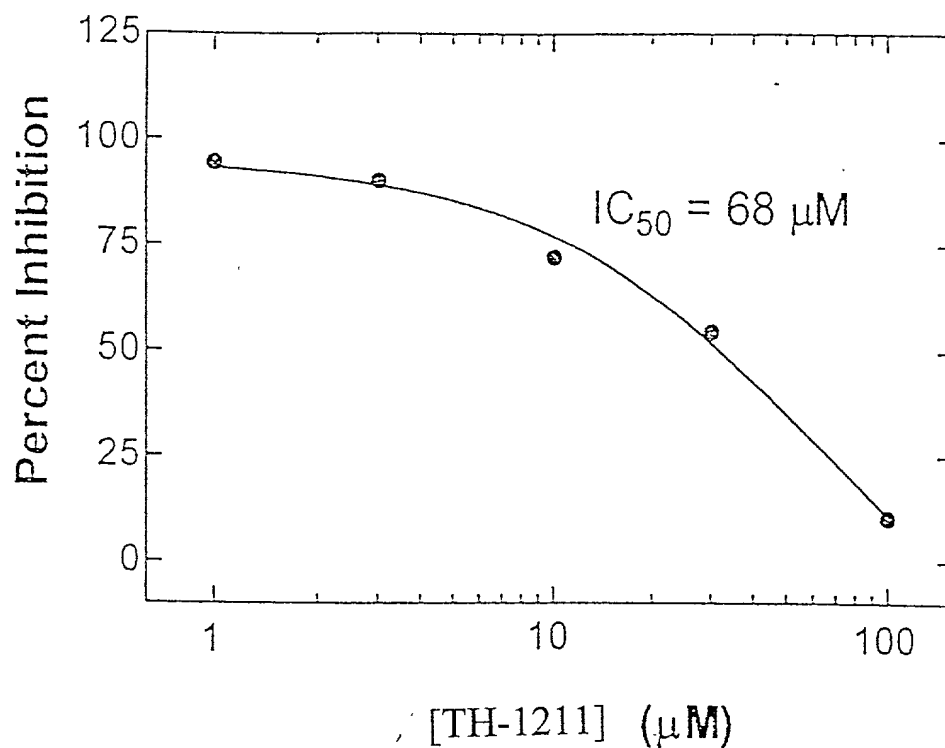
X Title	Data Set-A		
	Y	SEM	N
100	10.5	1.01	3
30	54 7	0.55	3
10	72.2	0.81	3
3	90 4	1.81	3
1	94.4	0.99	3

FIGURE 22

LNCaP/TH-1211

Percent Inhibition vs [TH-1211] (μM)

$IC_{50} = 2.7 \ \mu M$
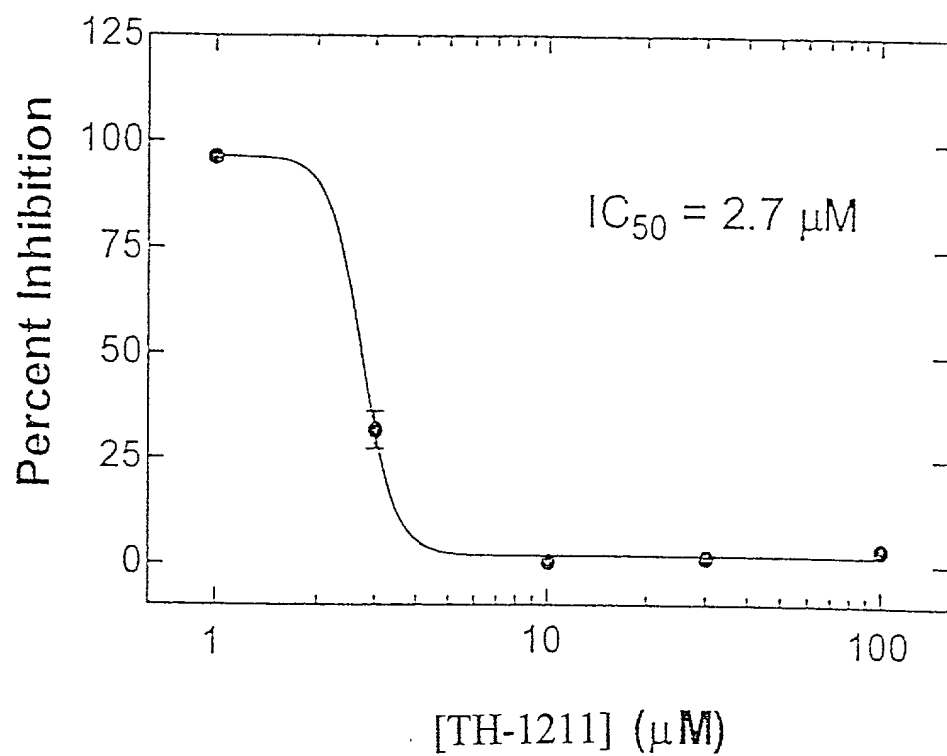
X Title	Data Set-A		
	Y	SEM	N
100	3 6	0.29	3
30	1.4	0 38	3
10	0 4	0.13	3
3	31.8	4.53	3
1	96.4	0.53	3

FIGURE 23

MDA-468/TH-1211

Plot: Percent Inhibition vs [TH-1211] (µM), with $IC_{50} = 54 \ \mu M$
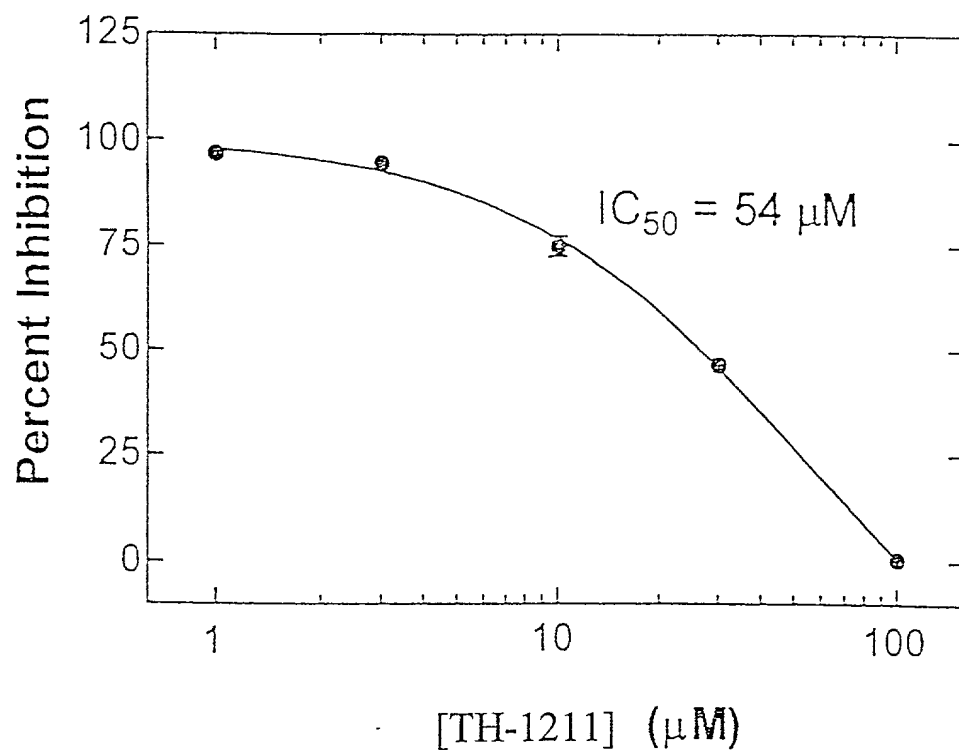
X Title	Data Set-A		
	Y	SEM	N
100	0.6	0.47	3
30	46.5	1.29	3
10	74.9	2.48	3
3	94.7	1.37	3
1	96.9	1.79	3

FIGURE 24

MDA-361/TH-1211
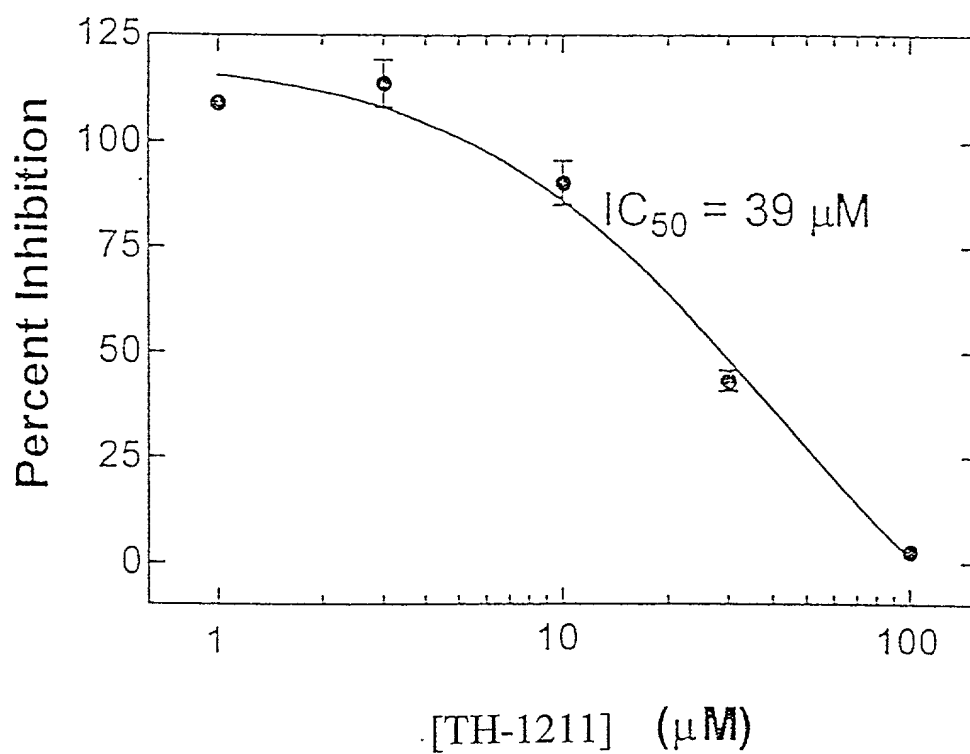
X Title	Data Set-A		
	Y	SEM	N
100	2 7	0 58	3
30	43.3	2.47	3
10	90.4	5 33	3
3	113.8	5.53	3
1	109 1	1.91	3

FIGURE 25

Jurkat / TH-1205

$IC_{50} = 8.8 \mu M$
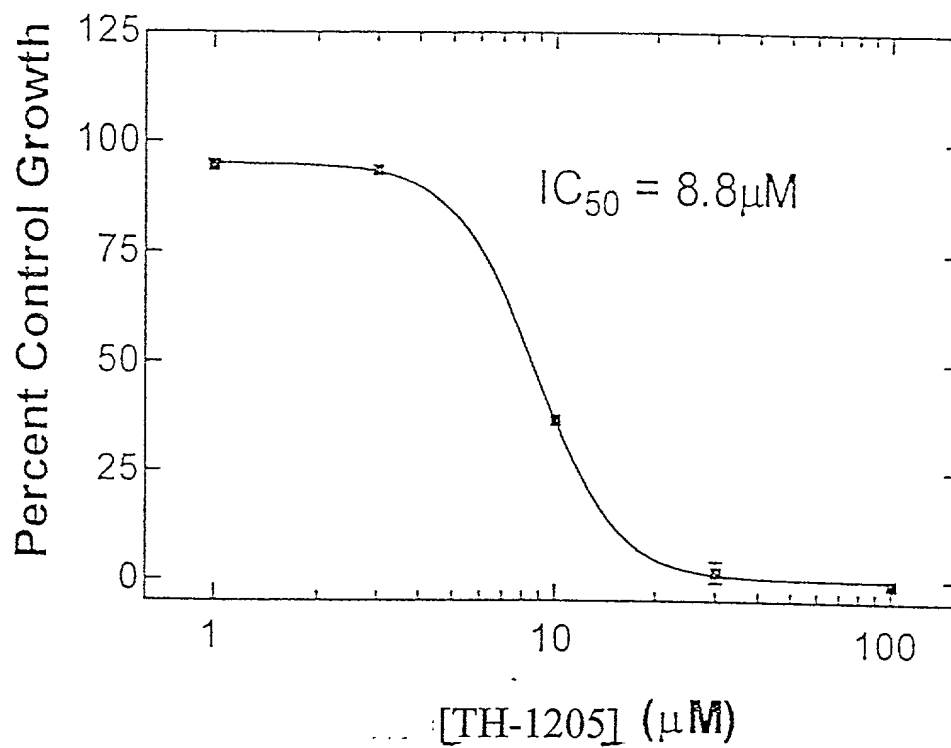
[TH-1205]	Data Set-A		
	Y	SEM	N
1	94.7	1.25	3
3	93.6	0.90	3
10	36.6	0.39	3
30	1.8	2.54	3
100	-0.7	0.19	3

FIGURE 26

PC3/TH-1205

IC$_{50}$ = 65µM
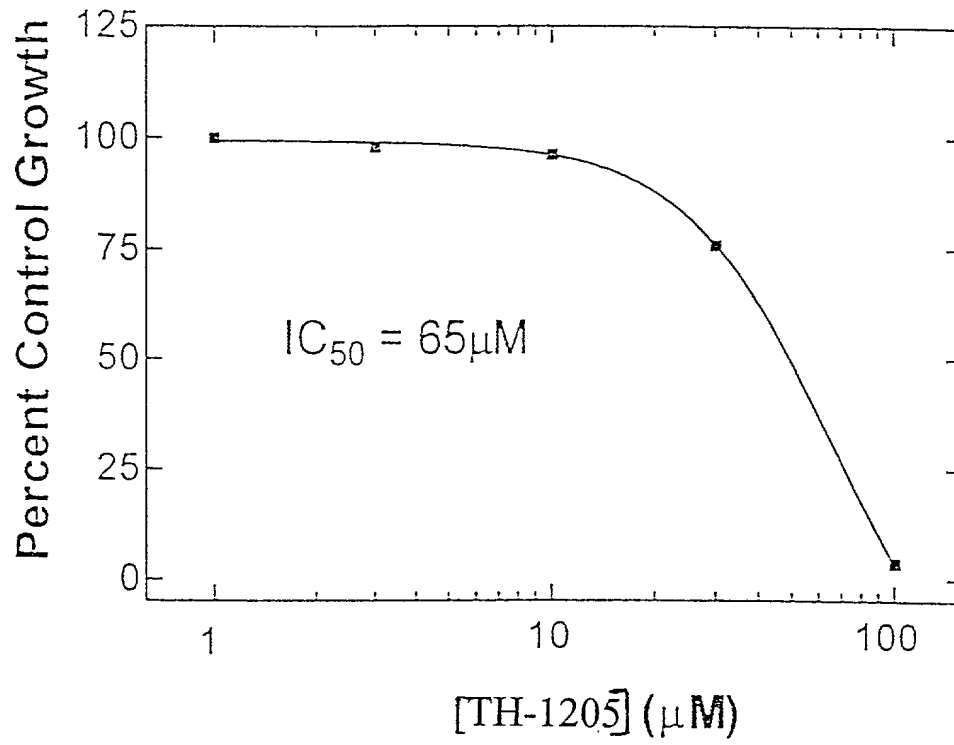
[TH-1205]	Data Set-A		
	Y	SEM	N
1	100.1	0.89	3
3	98.1	0.88	3
10	96.5	1.05	3
30	76.0	0.39	3
100	3 9	0.39	3

FIGURE 27

LNCaP/TH-1205
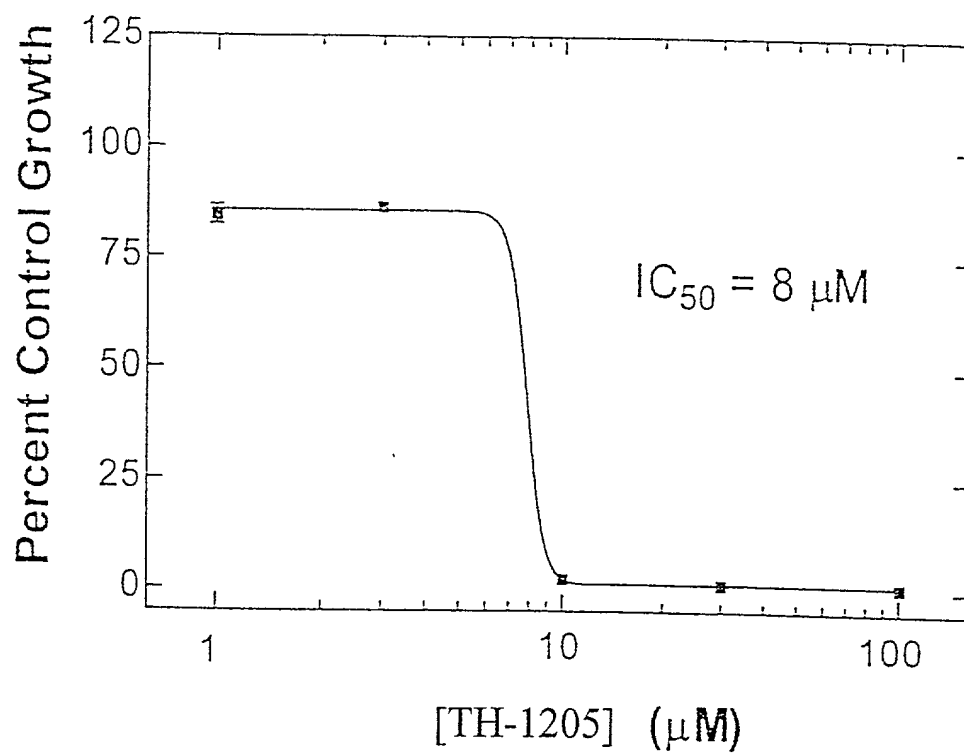
[TH-1205]	Data Set-A		
	Y	SEM	N
1	84.7	2.15	3
3	86.5	0.54	3
10	2.3	0.21	3
30	1.1	0.14	3
100	1.1	0.12	3

FIGURE 28

MDA-468/TH-1205
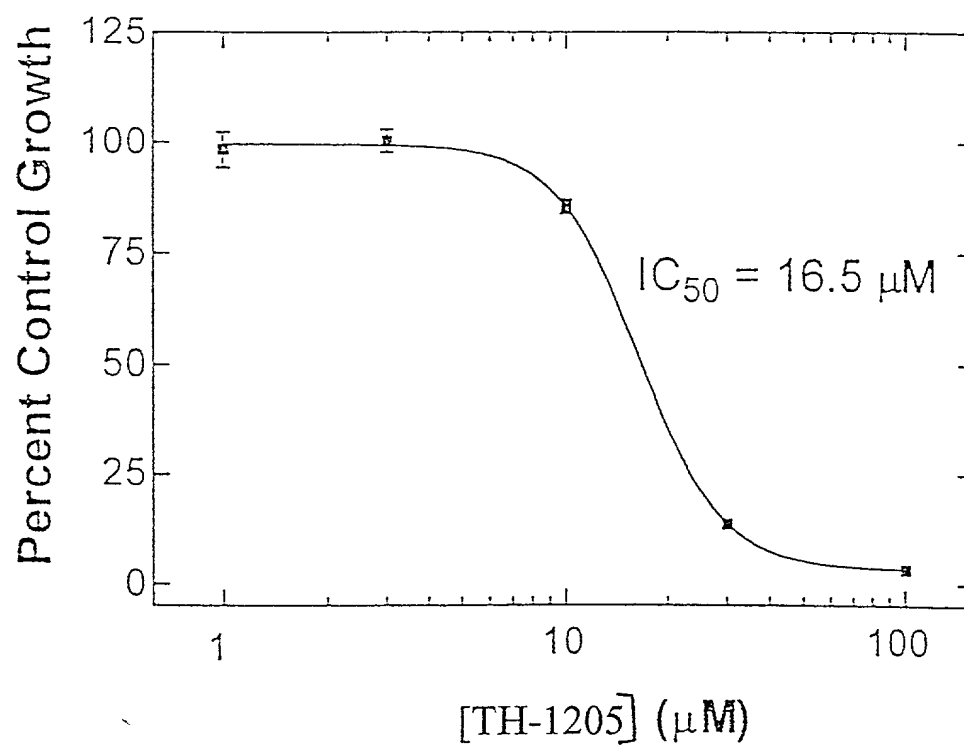
[TH-1205]	Data Set-A		
	Y	SEM	N
1	98.5	4.07	3
3	100.4	2.71	3
10	85.6	1.59	3
30	13.6	1.16	3
100	3 5	0.15	3

FIGURE 29

MDA-361/TH-1205

IC$_{50}$ = 11.9 μM
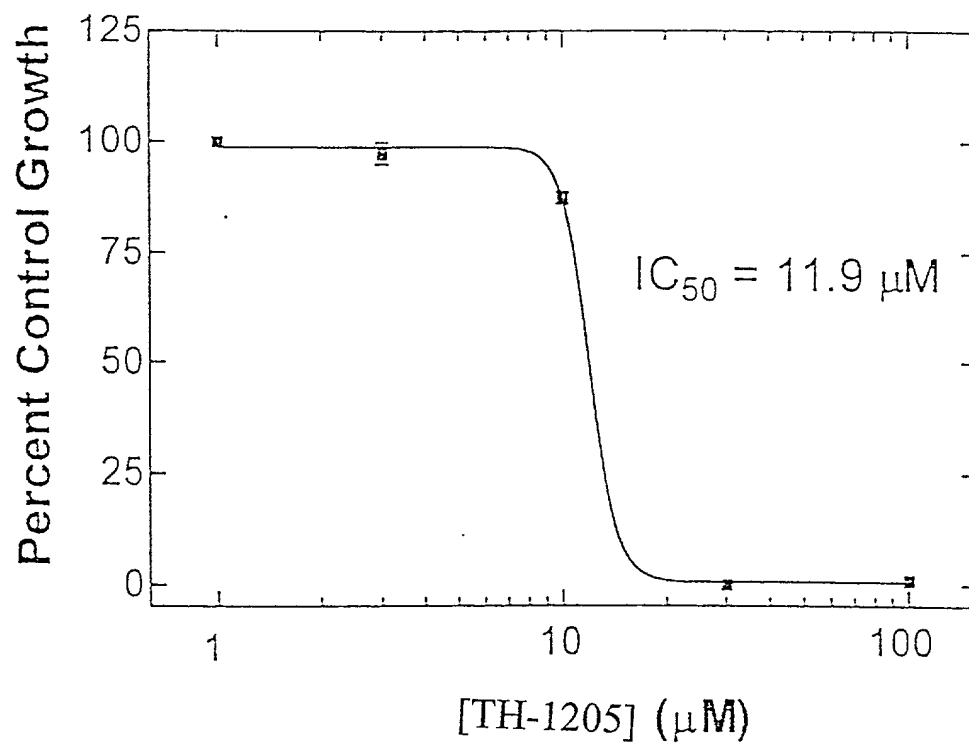
[TH-1205] (μM)

[TH-1205]	Data Set-A		
	Y	SEM	N
1	100 0	0.60	3
3	97 3	2.45	3
10	87 5	1.33	3
30	0 2	0.16	3
100	1.1	0 13	3

FIGURE 30

Jurkat/MMR-64

[MMR-64] (μM)
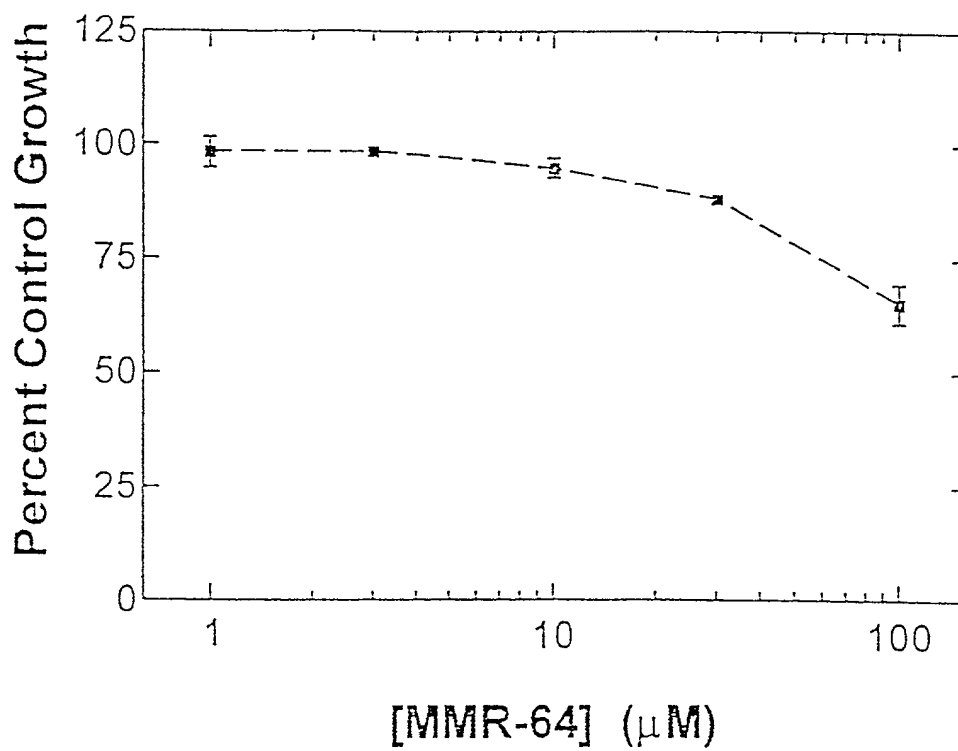
[MMR-64]	Jurkat		
	Y	SEM	N
1	98.4	3.31	6
3	98.5	0.37	6
10	95.0	2.11	6
30	88 3	0.13	6
100	65.4	4.24	6

FIGURE 31

PC3/MMR-64
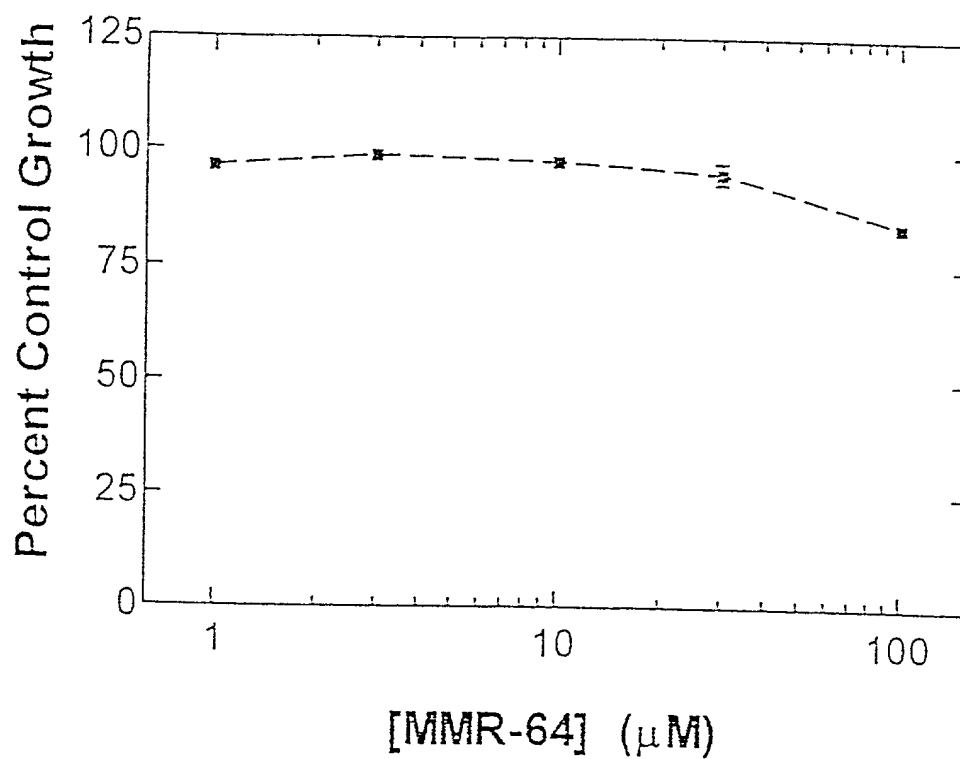
[MMR-64]	PC3		
	Y	SEM	N
1	96.5	0.50	3
3	99.0	0 32	3
10	97.9	0.61	3
30	95.6	2.28	3
100	84.4	0.43	3

FIGURE 32

LNCaP/MMR-64

IC$_{50}$ = 64.5 μM

Percent Control Growth vs [MMR-64] (μM)
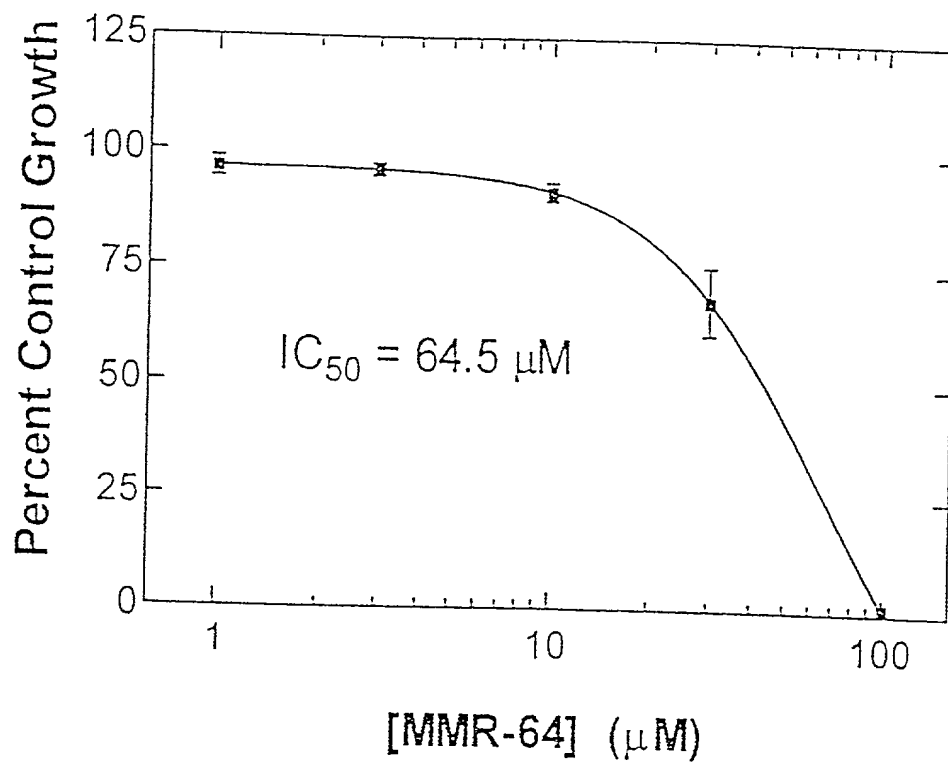
[MMR-64]	LNCaP		
	Y	SEM	N
1	96.5	2.19	6
3	95 9	1.30	6
10	91.7	2.00	6
30	68 3	7.41	6
100	1.4	0.42	6

FIGURE 33

MDA-468/MMR-64
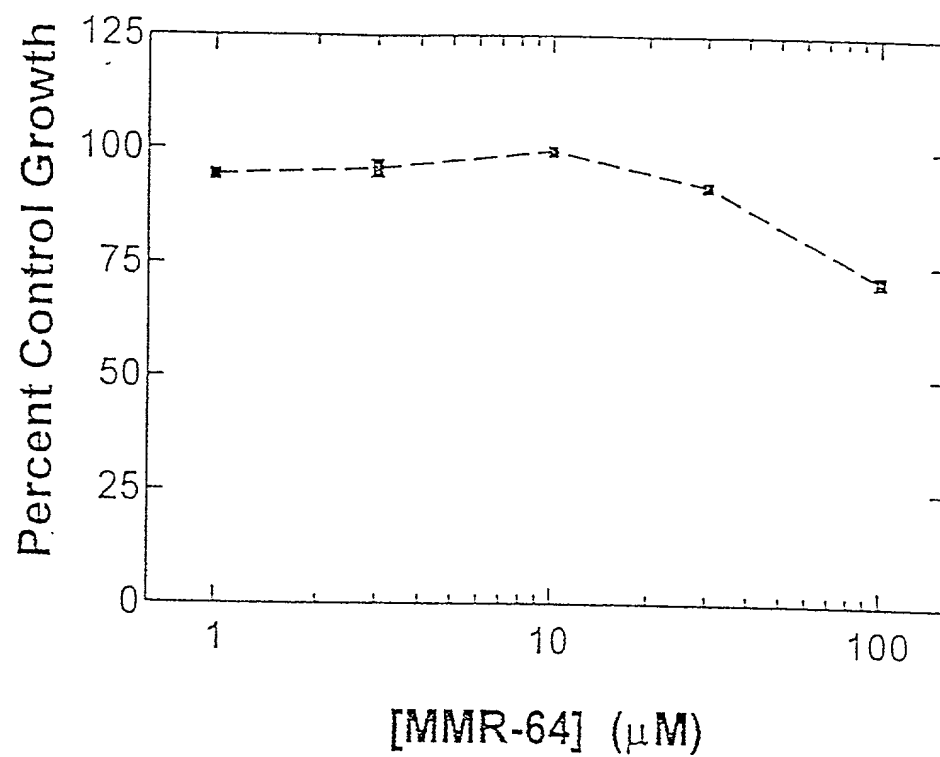
[MMR-64]	MDA-468		
	Y	SEM	N
1	94.6	0.77	3
3	95.9	1.76	3
10	99.8	0.64	3
30	92.4	0.96	3
100	71.7	1.40	3

FIGURE 34

MDA-361/MMR-64
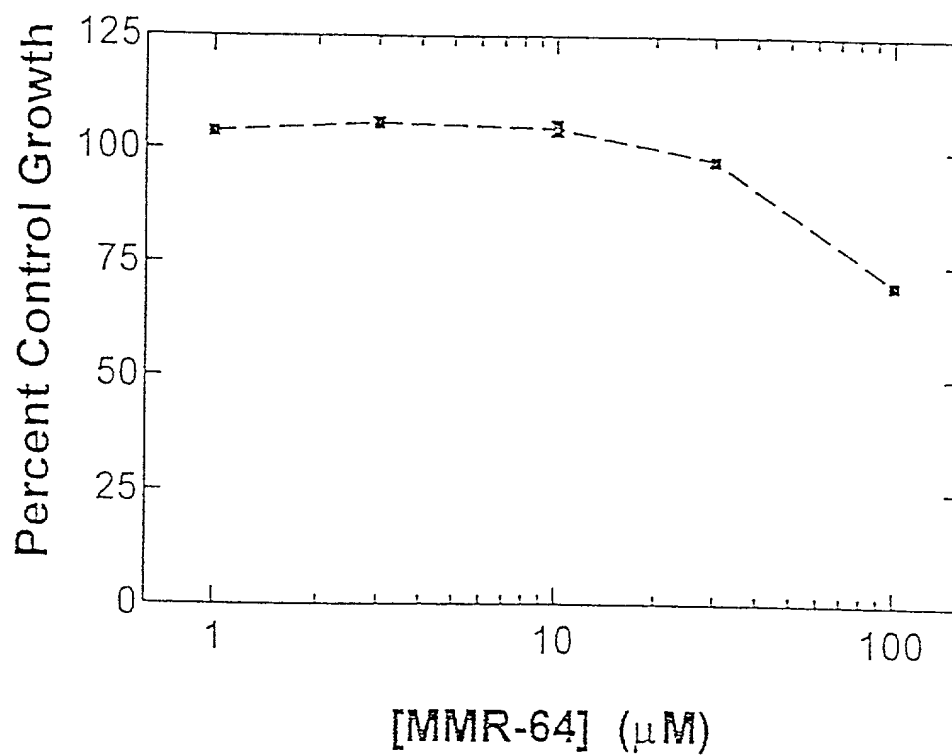
[MMR-64]	MDA-361		
	Y	SEM	N
1	103 7	0.71	3
3	105.6	1 20	3
10	104.5	1.62	3
30	97.7	0.90	3
100	70 8	1.00	3

FIGURE 35

Jurkat/MMR-70
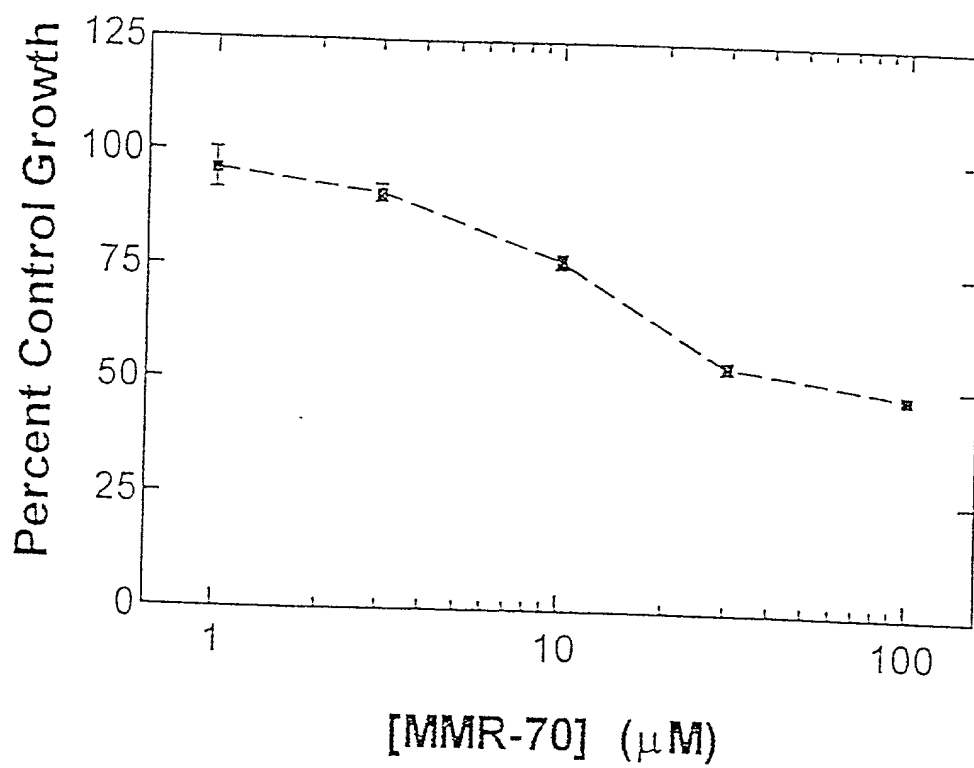
[MMR-70]	Jurkat		
	Y	SEM	N
1	96 3	4.37	6
3	91.2	1.92	6
10	76.6	1.60	6
30	53.9	1.27	6
100	48 1	0.42	6

FIGURE 36

PC3/MMR-70
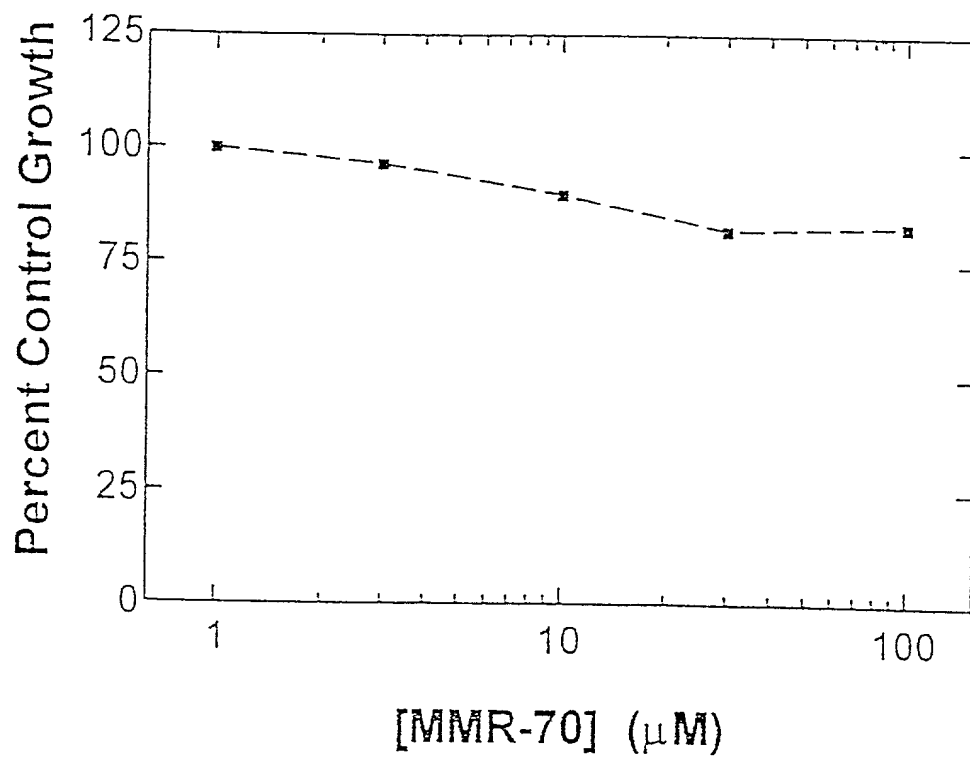
[MMR-70]	PC3		
	Y	SEM	N
1	100.0	0.93	3
3	96.6	1.13	3
10	90.2	0.71	3
30	82.3	0.30	3
100	83 3	0.44	3

FIGURE 37

LNCaP/MMR-70

$IC_{50} = 6.1\ \mu M$

[MMR-70] (μM)
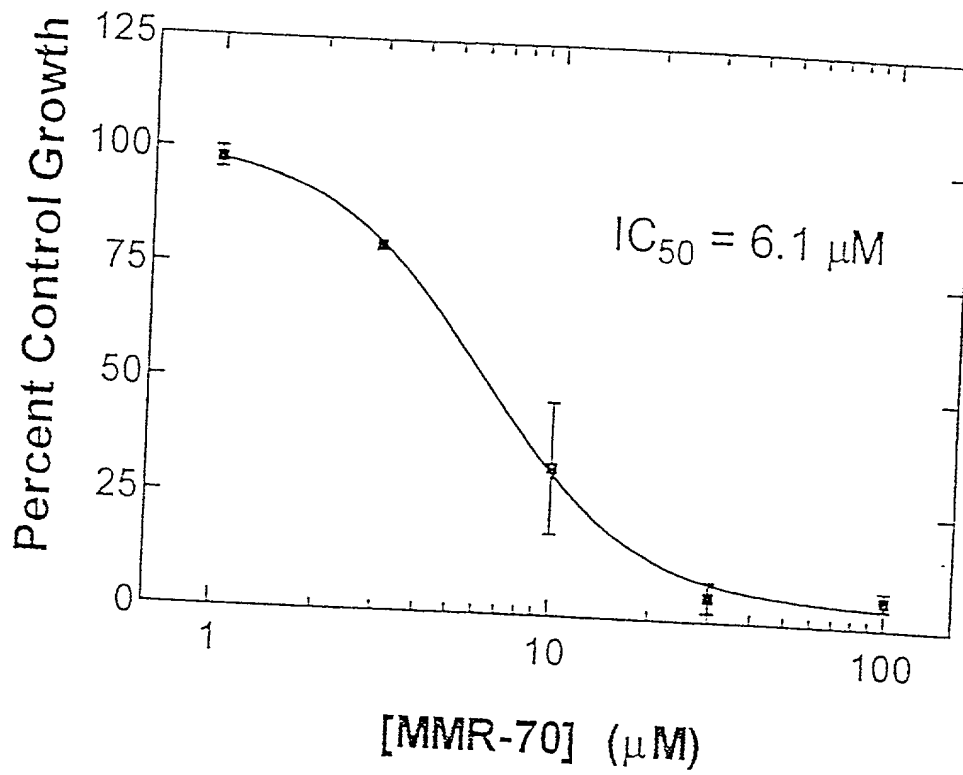
[MMR-70]	LNCaP		
	Y	SEM	N
1	98.1	2.35	6
3	79.9	0.14	6
10	32.4	14.34	6
30	5.5	3.47	6
100	6.4	2.03	6

FIGURE 38

MDA-468/MMR-70
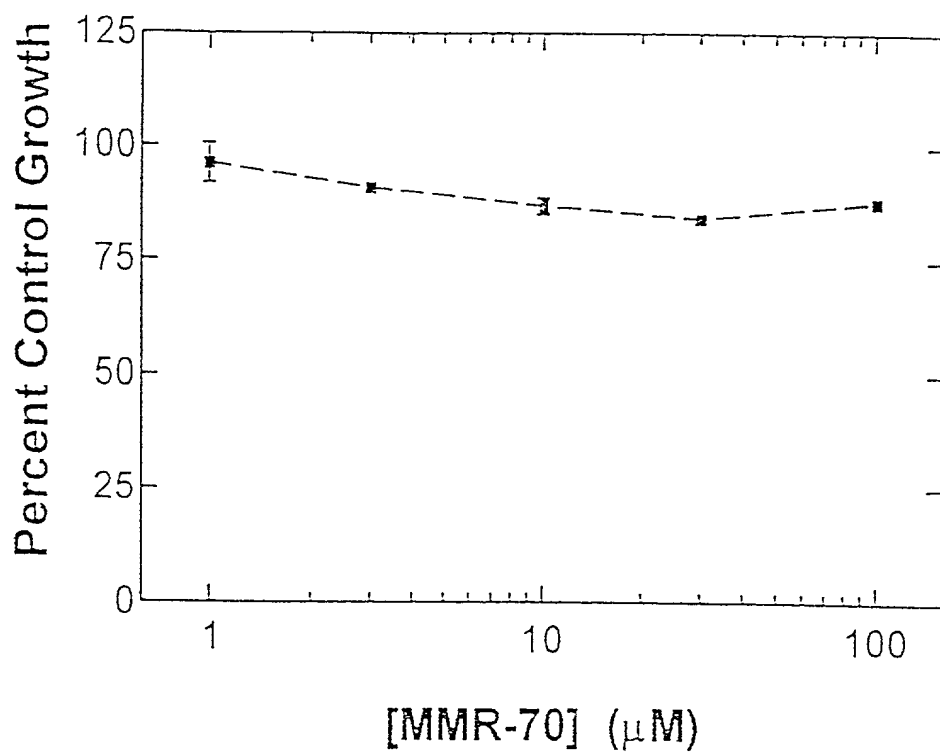
[MMR-70]	MDA-468		
	Y	SEM	N
1	96.4	4.34	3
3	91.0	0.12	3
10	87 1	1.72	3
30	84 5	0.68	3
100	88 0	0 40	3

FIGURE 39

MDA-361/MMR-70
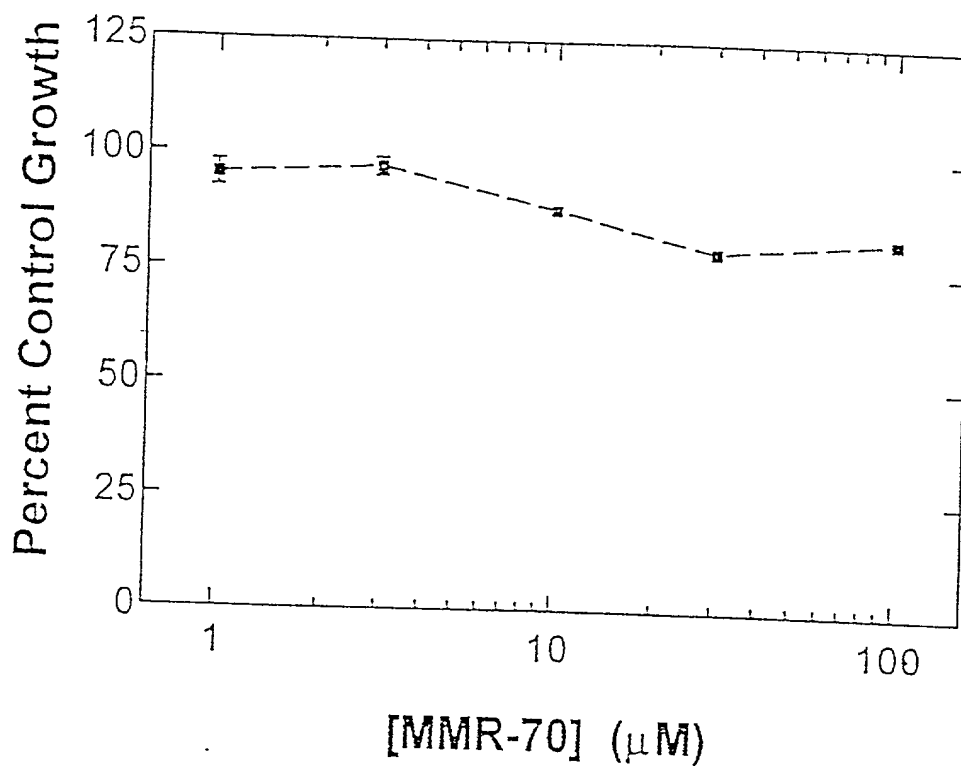
[MMR-70]	MDA-361		
	Y	SEM	N
1	95.5	2.78	3
3	97.2	1.96	3
10	88.2	1.07	3
30	79 5	1.06	3
100	82.6	0.71	3

Correlation of Ca²⁺ Influx and Proliferation

FIGURE 40

FIGURE 41

FIGURE 42